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SIR RONALD ROSS*

SIR MALCOLM WATSON

Ring out old shapes of foul disease,
Ring out the narrowing lust of gold,
Ring out the thousand wars of old,
Ring in the thousand years of peace.

As the bells of Christendom rang out the ninteenth century, they proclaimed two discoveries which were to save millions from "old shapes of foul disease;" for in India between 1897 and 1898 Ronald Ross discovered that malaria was spread by the anopheles mosquito, and in 1900, in Cuba, Walter Reed and his colleagues showed how yellow fever was spread by the mosquito known as *Aedes aegypti.*

You have honored me with an invitation to speak to you this evening in commemoration of the jubilee of Ross's discovery. He always insisted that his work was done not for the sake of science but for humanity, and I am sure nothing would have delighted him more than to hear that we have used his discovery with profit for that purpose. He was indeed a "Helper of the World and a Friend of Man"; and tonight I feel we stand at the bar of history to give an account of our stewardship.

So my main purpose is to tell you where and how we have used the discovery to triumph over malaria, and particularly of the six outstanding achievements; to tell where for long we were slow in starting, and why, so that we may be reminded of the danger that besets the discoverer and the new idea however beneficial it may be to mankind.

Before doing this I would briefly remind you of Ross's eight years of research before success came—and in the course of what I say you will form some picture of one whom Carlyle would have called a "Great Man", and "*The Hero as Scientist*"; of his youth before the discovery; of his career as a man after it. For nearly 30 years it was my privilege to call him friend.

THE YOUTH ROSS

As a medical student Ross was a failure. His interests were wider than the medical course, for as a youth "he determined to acquire some mastery of all the arts of man, and he did in fact acquire a mastery of mathematics and music, became a composer of songs, a poet of very great eminence, a painter of distinction, and a scientific researcher of the very first rank." That is how his friend Mr. John Masefield, the Poet Laureate, described him in 1936.

* An oration delivered before the Fourth International Congresses on Tropical Medicine and Malaria at a Special Meeting in Commemoration of the Fiftieth Anniversary of the Discovery by Ronald Ross of the Method of Transmission of Malaria, Departmental Auditorium, Washington, D. C., May 14, 1948.

Ross, describing these things in a discourse to the Royal Institution of London in 1920, declared:

"Do you really imagine that science is concerned only with the discovery of petty utilities; art with the discovery of new tricks of technique; and literature with mean books written by, for, and about mean people? . . . I say not art for art's sake, or science for the sake of science, but both for humanity."

Here he stated the guiding principle of his life, and he pursued his principle at all cost. Mr. Masefield said of Ross on the same occasion: "Ronald Ross was the most gifted, most beneficent and most forceful man it has ever been my privilege to know."

Time will not allow me to speak of any of Ross's many facets except the medical, but I have asked his publishers, Mr. Murray, and Messrs. George Allen & Unwin, to arrange for the exhibit here of some of his books; some of them are out of print. And also of Mr. R. L. Megroz's book, *Ronald Ross, Discoverer and Creator*, which will interest many, for Mr. Megroz wrote from personal knowledge of Ross. They will be found in the exhibition.

THE MALARIA DISCOVERY

For four years Ross worked on wrong lines and "fell into error," as he tells us. Then in 1895 he met Patrick Manson, a Scot despite his Christian name. Manson, the "Nestor" of the younger workers in tropical disease, explained to Ross his version of the idea that mosquitoes spread malaria. He believed, as a result of work he had done in China in 1878, that the mosquito became infected with malaria when it bit a man with that disease; that subsequently it died on water; and that man became infected when he drank the water. Manson advised Ross "to follow the flagellum" when he returned to India, for he correctly believed that this "flagellum" was the form of the malaria parasite destined to infect the mosquito. Unfortunately the "flagellum" behaved like the giraffe when it said to the leopard as he moved into the forest, "Now watch. One-two-three—and where's your breakfast?"

It was two long years before Ross discovered what had happened to the "flagellum," for it was even better camouflaged than the giraffe. He was looking for a fine colorless thread quivering its way among blood cells. What he found on the 20th of August 1897 among the fibres of the wall of the mosquito's stomach was a tiny cell with little black spots like little beady eyes staring up at him with not the quiver of an eyelid. He recognized it as the malaria parasite. He called the 20th of August, "Mosquito Day." That night he added to his poem *In Exile* the following well-known lines:

This day relenting God
Hath placed within my hand
A wondrous thing; and God
Be praised. At His command,
Seeking his secret deeds
With tears and toiling breath,
I find thy cunning seeds,
O million-murdering Death!

His work was now interrupted for the second time. There was some delay before

Manson persuaded the India Government to put Ross on special research on malaria in Calcutta. With the key to the problem in his hand, he finished the research in a few months, and on the 9th of July, 1898, showed that the malaria parasite, after strange developments in the mosquito, returned to man as it had come from him, through the bite of the insect.

TRIUMPH

The measure of Ronald Ross's triumph is in the record of what men have done since with the power he put in their hands. In the six outstanding achievements of which I shall speak, the work of Walter Reed on yellow fever and the work of Ronald Ross on malaria came to fulfillment.

Havana and Panama

After the two discoveries that malaria and yellow fever were carried by mosquitoes the United States Government was "quick off the mark," first in Havana in 1900, then in Panama in 1904 and in continental United States of America in 1912.

In 1913, I spent about 3 weeks on the Canal Zone, and walked over the whole area under sanitary control. Major (now General) Noble was kind enough to accompany me on many occasions or arrange for an inspector to go with me. Whatever I wanted to see I was shown, and there was the frankest discussion and criticism of their own work by the department of sanitation. Each night I made careful notes of what I saw and embodied them in a book—*Rural Sanitation in the Tropics.*

At Panama between 1881 and 1889 the French had died of yellow fever and malaria as if mown down by machine guns. In 1913 there had been no case of yellow fever for 7 years and malaria was represented by about one-half of 1 percent of the labor force per week. The labor force was as healthy as if they had been living in a temperate region, and the greatest engineering work the world had seen was moving smoothly to its near completion. So I feel there is some justification for the remarks I am about to make and the conclusions I drew:

First: That William Crawford Gorgas was the greatest sanitarian the world had seen.

Second: His was pioneering work.

Third: Becoming a commanding general, he had been on active service in the deadliest of campaigns, first in Havana in 1898, then in Panama from 1904 to 1915, and in the United States, South America, and Europe until his death in London on the 4th of July, 1920. I know of no medical man who has borne so heavy a burden for so long a time with such uniform and complete success; not always with the support he was entitled to expect.

Fourth: He never lost a battle.

Presenting Gorgas for an honorary degree at Oxford in England, the orator said:

"The reputation of Gorgas as a scientist has been challenged in certain quarters, in view of the fact that he was not responsible for the actual discoveries without which his work could not have been done. For this he needs no defense. Science and art are at their greatest when they join hands, and the man who acts as a link between discovery and its application needs a combination of qualities as rare as those of the pure investigator. It has been too much the pride of the seeker after abstract

truth in the exact sciences to care little as to its application. But even when research has been undertaken with the sole aim of finding the cause of an epidemic fever or the source of an infection, the successful investigator would often cut a poor figure as the organizer of an expedition to stamp out the scourge in the light of his discoveries. It is not only as a scientist but as a leader of men, as the hero of at least two of the most successful campaigns ever waged, that the name of Gorgas will always be gratefully remembered."

Honour to whom honour is due.

In Havana Gorgas found in Dr. Henry Rose Carter, an officer of the United States Public Health Service, not merely a scientific adviser but a lifelong friend. When Gorgas had made Havana perhaps the cleanest city in the world, yellow fever mocked his efforts and slew with cunning discrimination those in the cleanest houses in this cleanest town. There, where science and art of sanitation had proved a 100 per cent failure, it was Carter who aided Gorgas in turning to attacking the mosquito as well as screening the sick. After Havana, Carter went as chief assistant to Panama, and later commenced the control of malaria in the United States.

An army requires more than a general staff; there must be good field officers. First in the Mission at Havana, and later in Panama, Gorgas gave Le Prince the task of field organization and supervision in developing the attack against the mosquitoes of yellow fever and malaria. Subsequently at Carter's insistence he became the chief field organizer of malaria control in the United States in 1914. In Panama Gorgas gave Le Prince the title of "chief sanitary inspector," a title he has adorned with imperishable laurels. Joseph A. Le Prince, the son of a distinguished French inventor and a Yorkshire mother, graduated in civil engineering at Columbia University, New York, in 1898, and joined Gorgas in Havana in 1901. He cleared yellow fever out of Havana in a matter of months in 1901, for the first time in its history since 1762.

His chart of the number of mosquitoes swept up from the floors after fumigation convinced Le Prince the victory had been won for good. But Gorgas feared that yellow fever would stage a come-back and he did not want to be caught unprepared. So he hesitated to disband Le Prince's organisation. The difficulty was overcome when Le Prince suggested he might wipe out malaria while they waited to see what happened about yellow fever. So Le Prince cleared malaria out of Havana with equal success and speed, although the technique of destroying the mosquitoes carrying the two diseases is totally different.

It was all pioneering work, but Le Prince had the essential qualities—imagination, invention, energy, organising power. Like Admiral Nelson, he had a "blind eye" when a job had to be done in a hurry without approval from above. He had also an insight into the minds of mosquito and man. Le Prince knew that yellow fever did not automatically disappear when a town in the American tropics got a piped-water supply—very much the reverse. For he knew what the women of Havana knew—that a woman's hair looked better and kept looking better when washed in rain water than when washed in pipe water; and they were ready to fight for woman's right to look her best!

At the Seventh Congress of the Far Eastern Association of Tropical Medicine held

at Calcutta in 1927 resolutions were passed on the control of malaria. Among these was the following:

"The Congress desires to stress the need not only of thoroughly trained malarial research officers, but of expert malarial engineers in whichever type of malaria prevention is at stake."

I have heard with pleasure that a university has conferred the degree of doctor of science on Joseph Augustin Le Prince—the pattern and exemplar for sanitary engineers in the Tropics. It is a pleasure to record that he continues in retirement his stimulating leadership and has used the inspiring story of Reed and Ross in developing an active high school organization in the middle Mississippi Valley, the Howard-Krause Society,* which uses as its motto Le Prince's oft repeated "It can be done."

But it was not all smooth sailing for either Gorgas or Le Prince.

CRISIS IN THE WEST

"I am sorry for you tonight, Mr. President," wrote his friend Dr. Alexander Lambert to President Theodore Roosevelt in 1905. "You are facing one of the greatest decisions in your career. Upon what you decide depends whether or not you are going to get your canal. If you fall back on the old methods of sanitation you will fail, just as the French failed. If you back Gorgas and his ideas, and let him make his campaign against mosquitoes, then you will get your canal. I can only give you my advice; you must decide for yourself. There is only one way of controlling yellow fever and malaria, and that is the eradication of mosquitoes. But it is your canal; you must do the choosing, and you must choose tonight whether you are going to build that canal."

It was a critical moment for the canal. The Canal Commission had recommended that Gorgas should be dismissed and "replaced by a man with more practical ideas."

To add to the President's difficulty, Gorgas' dismissal was supported by the then Secretary for War.

With my own ears I heard Gorgas tell that to a great congress of physicians and surgeons here in Washington in 1913.

Indeed it was a critical moment for more than the canal. The wrong decision would have set back the control of yellow fever throughout the Western Hemisphere, and might have led to its spread to Asia. When yellow fever struck Memphis, Tenn., in 1878, it killed 4,200 out of 6,000 whites between the 16th of August and the 27th of October. The life of the city was paralysed, and all fled who could. In Asia there are more than 800 million nonimmune people, and there is no reason to think that their mortality would be less than that of Memphis in 1878 were a yellow-fever epidemic once started. Well might Sir Patrick Manson describe an outbreak of yellow fever in Asia as a world disaster of appalling magnitude. There is still a danger to Asia from yellow fever spreading from Africa, as I pointed out officially to the Government of Malaya in 1914 and said in my *Rural Sanitation in the Tropics*, published in 1915.

The President made the right decision. Gorgas remained, and was promoted to membership on the Canal Commission.

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Rockefeller Foundation

The world has reason to recall with gratitude the names of many great citizens of the United States of America.

In creating the Rockefeller Foundation and the International Health Board, Mr. John D. Rockefeller planted a tree of life and "the leaves of the tree were for the healing of the nations." It would take volumes to record all its deeds of mercy. Here I can speak only of its work against malaria. In addition to that done in the southern States of the United States of America; it taught Europe and the Malaria Commission of the League of Nations that it was cheaper to prevent malaria than to cure it, and the cooperation of Hackett and Missiroli claared malaria out of the Roman Campagna where it had held sway for 2,000 years.

Its high-water mark was in eradicating *Anopheles gambiae* from Brazil and from Egypt. There is nothing more brilliant in the history of the prevention of malaria than that described by Soper and Wilson in the book *Anopheles gambiae in Brazil, 1938–1940.* They threw this African invader out of Brazil and saved the whole Western Hemisphere. It prevented the deaths of millions of people, which the spread of this winged terror would have made inevitable. Indeed, it stands out as one of the greatest sanitary achievements of all time.

And now we must turn to India, the birthplace both of Ross and of his discovery.

CRISIS IN THE EAST

It was sheer tragedy that on the 15th of December 1898, the Director General of the India Medical Service had no friend to write:

"I am sorry for you today, Dr. Harvey. You are facing one of the greatest decisions of your career. Upon what you decide today depends whether or not millions of your fellow men will live or die. The decision must be yours. You must make it today."

He made the wrong decision.

You may call the story an epic, if you like. To me it appears as a major tragedy—with the dramatic unities in proportion.

Place: All the World's a stage:

Time: A lifetime—ours.

The cast: were men and women whom many of us knew:

The story: no mirror of nature, but the very substance of which drama is begotten—human life itself.

And yet here none of the darker sins and passions of men and women, lust, jealousy, ambition, murder set in motion events, which gathering speed, have involved the actors in disaster and death.

The curtain rises on a Calcutta Office in the year 1898. A "mild and ineffectual man" doing his duty to the best of his ability, I have no doubt. But he was not of the calibre of Directors-General of the Indian Medical Service which has done such brilliant work for India. In time and mind Harvey was 2000 years behind the Indian Emperor Asoka.

He congratulated Ronald Ross on his work; regretted he could not promise him

further work on malaria in India; was afraid he had no time to visit his laboratory. The interview lasted 3 minutes.

Ross left India on the 24th of February 1898, weary and worn from his long researches. But the cool weather and rest on the voyage revived him and he thought as he sailed along the sunny Spanish shores:

"In two years we shall stamp malaria out of every city and large town in the tropics —at least if they possess sanitary departments as in British possessions. And this is not the dream of a visionary. My experience of sanitation in Bangalore has taught me what few medical men possess, a thorough knowledge of town management, and I knew what I was talking about—sanitary organisation, town cleansing, sanitary engineering, houses, yards, sewers, official procedure, and the rest of it."

He had the further qualification of having studied and taken the diploma of public health of London, and having studied the new science of bacteriology under Klein on his leave in 1880. The voyage had revived his hope, and hope like

> "*. . . love resembleth the uncertain*
> *Glory of an April day, which*
> *Now shows all the beauty of the sun, and*
> *By and by a cloud takes all away!*"

How descriptive of Ross' life Shakespeare's words are—with its hope and despair, its joy and sadness, its tragedy, triumph and defeat. But thank God, it was triumph before the end.

On his arrival in England in March 1898, he took a poorly paid appointment at Liverpool instead of starting practice in London, so that he might continue his research in malaria and make a start on prevention. In the same year, he confirmed his discovery in the African *Anopheles* at Sierra Leone, and on the 2d of July, 1901, started a demonstration of mosquito control there with money provided by Mr. James Coats, Jr., of Paisley, hoping the Government would continue the grant when his money was finished. But it did not do so, and some time later said his work had been a failure.

In 1902–4, a Commission appointed by the Royal Society of London and the Government of India conducted an experiment, but "were unable to demonstrate that anopheline mosquitoes and malaria could be appreciably reduced by the new method in Mian Mir." The place of this experiment was near Lahore, India. The experts in India concluded that antilarval operations were "difficult," "ineffectual," "useless," and "futile."

The one man essential for success—Ronald Ross with his practical experience—was 6,000 miles away; nor was there an engineer like Le Prince of Panama or Harold Gray of California in the team, or the result would have been very different.

In a letter to me in 1904, Ross wrote: "I fear that experiment will put back the hands of the clock in India for another generation." It was to do so, and not merely in India but in extensive regions of the world—for it appeared so authoritative.

In the next 20 or 30 years tragedy followed on tragedy in the British Empire and beyond for the lack of vision and the wrong decision made in the Calcutta office in 1898. I can give only a few examples.

In Macedonia, in 1917–18, there were 160,000 cases of malaria in the British Army. "Malaria dominated the military and medical situation," is printed in the *Official History of the War.*

In Ceylon, in 1934–35, a malaria epidemic killed 80,000 people. For 800 years malaria had made uninhabitable about one-third of the island, in which the remains of a great civilization lay buried under a mantle of green jungle.

When the last war broke out, the West African ports were as malarious as ever. So when the allied forces landed to make air bases for the Middle East, some 80 per cent of the men became infected. Brazil pointed out that these ports were a danger to the whole Western Hemisphere in exporting *Anopheles gambiae* and other pests; and she was entitled to do so, for had she not already thrown *A. gambiae* out of her own territory?

THE MAN ROSS

By 1904 his critics were in full cry. Their arguments were:

1. It was impossible to reduce mosquitoes.

2. Mosquitoes like Nature abhorred a vacuum—they would flow into any area in which they had been destroyed—if you had managed to do the impossible.

3. A daily dose of quinine would cure malaria, prevent the *Anopheles* becoming infected, and so end the disease.

4. Malaria was a social disease. With improved housing and better food the malaria parasite would do men no harm. As in the ending of the other fairy tales, they would live happily together ever after.

And all this in spite of the well-known facts—indeed, proved by Dempster's brilliant work in India 100 years ago—that malaria was a very local disease; also that well-fed and well-housed British and American soldiers in malarial regions suffered severely from malaria; and that successful mosquito malaria control had been done at Havana, Panama, Ismailia, Malaya, and elsewhere.

Although his genius had been recognized by almost every university and every learned society throughout the world, a number of wrong things were said of Ross after his death by those who did not know him personally. He was described as shy and timid, and one who hid those qualities under a mask of indifference; it was said that he was not made for commerce with his fellows; and even that he was not a scientist—this latter by a well-known scientist to myself.

So far from being shy, he was an easy and fluent speaker, and could hold an audience spellbound. In private life he was a genial host, an interesting talker, from his wide knowledge and extensive travels. He was most approachable, and to the younger generation ever helpful. When Dr. Harvey came to his laboratory and frankly admitted his mistakes, Ross forgave him.

On the other hand, he could not hear foolish arguments against mosquito control without exposing their folly; and injured pride made enemies. He could not suffer fools gladly, and in defense of the policy which meant so much to human happiness, he was belligerent and careless of the bitterness which his attacks created.

A trickster he never forgave; unhappily "the cunning keep the crown": for in England, as in Denmark,

"A man may smile and smile and be a villain."

Unhappily, too, his critics were in the inner lines. "After his discovery, the rest of his life was devoted to enlarging and completing what he had begun. It was passed in an obscurity which is likely to occasion surprise in the future as well as regret," wrote *The Times* on his death. "They will admire the innate pugnacity of the man as well as his genius, his patience, and his high moral courage. It is probable they will also supply him with his complete justification by carrying out on a large scale the measures he advocated so long and so earnestly. One thing is certain; namely, that Ross's service glows with an imperishable luster. He slew the dragon and delivered mankind from immemorial bondage."

For too long he did not recognise that in the one thing he regarded as important he was officially regarded as "unemployable," and "unemployment" had the usual result—financial disaster. When he sold his "archives" in 1925 the public realized something of the truth, and later a public subscription was raised to relieve his anxieties.

But the day of tribulation for the tribes of the Philistines was nigh.

"Fear not. Unsheath the naked falchion. Try
The end. For in the end, who dares deny
The utter truth will slay the utter lie."

R. R. 1890–93.

THE ROSS INSTITUTE OF TROPICAL MEDICINE AND HOSPITAL FOR TROPICAL DISEASES, PUTNEY, LONDON

A proposal to establish a Ross Institute, made in *The Times*, London, on the 23d of June 1923, was backed by many of the most distinguished men in every sphere of life. At the end of 1925 the Institute was opened, and under the wise guidance of Sir Charles McLeod, Sir Austin Chamberlain, Mr. A. Chester Beatty, and Sir Eric Macfadyen as chairmen in succession, and a standing committee composed largely of businessmen, it quickly made its influence felt in India; Ceylon; East, Central and West Africa; and elsewhere. Since Ross's death it has had two directors, myself until 1943, and Dr. G. Macdonald since. An important contribution to its success has been the Industrial Advisory Committee, which meets in the city of London for the convenience of its members. Its proceedings, which are widely circulated to the press as well as to its members, set out that "The Ross Institute Industrial Advisory Committee was formed in 1928 to keep Industry in touch with Science to make the Tropics Healthy, and to Expand the Markets of the World."

It has been fortunate in its chairmen, Mr. A. W. Still, a past president of the Institute of Journalists, Mr. G. H. Masefield, a brother of the poet laureate, and Mr. A. Wigglesworth, a leader in the African sisal industry. Of great value, too, has been its Malaria Course for Laymen. Nearly 1,000 men from many parts of the tropics, and of every occupation, took this course between 1928 and 1938.

Such was the success of the Ross Institute in its work overseas that it received and accepted in 1933 a proposal for amalgamation with the London School of Hygiene and Tropical Medicine—itself founded by a munificent gift from the Rockefeller Foundation. The Ross Institute of Tropical Hygiene, its new name, continued its work in the tropics and took over the teaching of tropical hygiene at the school.

Hand in hand, the Ross Institute and the Rockefeller Foundation, nonpolitical

and nonpartisan, have been welcomed by kings and princes, by governments, by great tropical industries, and by societies of peasants and humble folk. Today they are working in most tropical and nontropical countries where malaria is a curse, spreading the gospel of help and self-help, by precept and example, as Ross and Reed and all great discoverers and benefactors of mankind would desire. Nor have their achievements been mean.

Malaya

In 1926, Ross visited Malaya. I had the pleasure of driving him for hundreds of miles and showing him work for the prevention of malaria. He was acclaimed and fêted everywhere, for the people of Malaya, official and unofficial, had seen the benefit of malarial prevention over ever-expanding areas since its beginnings in 1901. By 1920, 100,000 lives had been saved in Malaya. On returning to London Ross reported to the committee that the work in Malaya was "the greatest sanitary achievement ever accomplished in the British Empire."

Not only was Malaya advanced in sanitation, but many tributes have been paid to its wise administration. At a meeting at Klang on the 5th of August, 1927, over which I presided, the distinguished Indian poet and Nobel Laureate, Dr. Rabindranath Tagore, said: "Last time when he came to this peninsula what struck him most was the fact that here they had a great many races who were living happy and contented lives in spite of their differences of language, religion and culture. They were together in the same neighbourhood and they had genuinely friendly feelings for one another. It seemed to him like the first sketch of a great picture in process of development. The people had come together, it might be for the sake of profit, for making money, for their utilitarian purposes, but the first step had been taken. The soil was fertile for their happy life, which was necessary for the cultivation of the friendly feeling. . . ." He also referred to the value of science.

In 1875 the British entered Malaya at the invitation of the Sultans to stop civil war and piracy.

From Malaya, Ross travelled to Calcutta. There a Memorial Gate at the Presidency Hospital, where he had completed his discovery in 1898, was opened by H. E. Lord Lytton, Governor of Bengal, after an interesting address by Sir John Megaw—later the distinguished director of Ross's old service.

Africa

Ross was greatly interested when, in 1929, the Ross Institute began work on Mr. A. Chester Beatty's group of copper mines in Northern Rhodesia, for he had not forgotten the neglect of his work in West Africa. Mr. C. R. Harrison, originally a rubber planter in Malaya, organized the antimalarial work, mainly by drainage and oiling, producing an immediate effect on the sick rate and death rate.

When I visited Rhodesia in 1930, a senior government medical officer said that as a mosquito could fly 5 miles and one mosquito could give malaria, mosquito-malaria control was impracticable. H. E. The Governor, formerly a medical officer in government service in West Africa, said to the managing director, Mr. A. D. Storke, and myself, that we were attempting the impossible. Two years later, Mr. Harrison's

work had converted the Governor, and when Mr. Storke and I met him again he said so.

In this part of Africa—600 miles from the Equator—sanitation on the mines included the control of the two great African carriers of malaria, *Anopheles tambiae*, breeding in pools, and *A. funestus*, breeding in the extensive swamps lying within and adjacent to the mines and their houses. In addition, the half-starved laborers who came from mud huts which they shared with a whole host of animals and parasites—fleas, lice, ticks, rats, mice, and snakes—brought with them many of the diseases which had been a scourge in medieval Europe. Within a short time a medical department, complete, competent, and combining curative and preventive medicine, had such an organization that the imported diseases did not spread and the population was as healthy as if they lived in a temperate region. Very remarkable, was how the African women rose to their new surroundings—a garden city, as I described it in a special article in *The Times*, London, on February 10, 1940: "But already the copper mines have shown the African what a better standard of life means, have stimulated the woman to seek it for herself and her family, and, not least important, have taught her to live it."

At a recent meeting of the Royal African Society in London there was a rather inconclusive discussion on the problem of incentives and how to induce the African to combine many tribes speaking different languages and with different religions and cordially disliking each other; how to get him to work harder, improve his farming, earn enough to pay for social services, instead of having for his first objective getting rid of the "master race." One administrative officer said that government activities in Africa had been along three lines, the first political, the second welfare, and the third economic, and they had been taken in that order. With all due respect I would suggest that this is the wrong order; that a lesson be taken from Malaya; that the copper mines should teach to all in Africa less of an inferiority complex to pests and parasites; and that the whole African social structure should be built on a "healthy village" such as I suggested in a paper before the Institution of Mining and Metallurgy, London, in 1942. In 47 years travelling in the Tropics I have not seen any people making a success of life, or exhibiting any surplus energy, who were sodden with disease. And it seems to me that to expect it from the African can only come from never having seen such a change in a man's physique and energy as occurs on the copper mines after a year's residence or on an estate in Malaya. I commend these matters to those responsible for the African Continent.

India

In 1930, a Branch of the Ross Institute was founded in India through funds provided by Sir Charles McLeod, and his friends, Dr. G. C. Ramsay was placed in charge. Brilliant results followed Dr. Ramsay's scientific and practical organisation. I can only summarise them. The health of Europeans and Indians improved; wages and profits increased; 600 young Indians were trained as malaria surveyors; antimalarial work was stimulated throughout India. When war came the tea-garden doctors trained by Ramsay supplied most of the malariologists required by the British Army for service from West Africa to Singapore. Many of them received decorations from

His Majesty the King. Ramsay received the Kaiser-i-Hind Gold Medal and later the Companionship of the Most Eminent Order of the Indian Empire. For his services with Lawrence in Arabia in the 1914–18 war he was made an Officer of the Most Excellent Order of the British Empire (Military Division). He retired in 1945, and has been succeeded by Dr. Alan Gilroy, one of my pupils and a tea-garden doctor, who, as a lieutenant colonel in the Royal Army Medical Corps, cleared Lagos, one of the West African ports, of malaria.

I have described what I regard as the six outstanding examples of malaria prevention by Ross's policy of mosquito reduction in the tropics—Havana, Panama, Brazil, Northern Rhodesia, India, and Malaya. They may be compared to the advantage or disadvantage of one another as the critic may be biased. In truth they are complementary and confirmatory, each has developed on the lines best calculated to achieve its object in its own peculiar circumstances. They represent different stages and phases in the work of stamping out disease in the tropics, and making these lands, many most fertile, and with great mineral wealth, pour forth their abundance for the benefit of mankind, instead of remaining the haunt of a few miserable and unfortunate human beings.

Thank God, these six brilliant achievements do not represent the total use made of Ross's discovery. In the last 10 years or so there has been a great expansion of the work, so that in this Jubilee year

THE SUN NEVER SETS ON IT

You see it in the southeastern States of the United States of America, where the work of the TVA is an outstanding achievement in conserving and using water for navigation and agriculture rather than running it "down the drain" as a waste product into the sea. There is work in the West African ports, in Holland, in Portugal, in Spain, in Italy, in Greece, in Cyprus, in Egypt, in Iran and Iraq; in India by the governments, the princes and the municipalities; in Ceylon, where there are schemes to reclaim the land so long abandoned to malaria; in Burma, Malaya, the Dutch East Indies; in Borneo; in the Philippine Islands where Dr. Paul Russell worked (as well as in India and Malaya) before he earned the Legion of Merit for his service with the Army in South East Asia, North Africa and Italy, and with his colleagues wrote his great book *Practical Malariology*. In California, Herms and Gray began as far back as 1911. Their work and experience were invaluable in the war, and form the basis of their book on *Mosquito Control* which must find a place in every library.

That is the account of our stewardship.

SUNSET

Ross did not live to see all these developments—the conclusive proofs of his wisdom. He died on the 16th of September 1932. For me, although it was the break of a long friendship,

"Whatever way my years decline,
I felt and feel, tho' left alone,
His being working in mine own,
The footsteps of his life in mine."

Ronald Ross Exercises—4th International Congresses on Tropical Medicine and Malaria

;Sir Malcolm Watson, who delivered an oration on Ronald Ross, Prof. George McDonald who succeeded Sir Malcolm Watson as head of the Ross Institute and Dr. Paul. F. Russell, Program Chairman.

Taken at the Ronald Ross Exercises

Lady Watson, Dr. N. H. Swellengrebel, Holland, Dr. H. E. Shortt, England, Dr. Hackett, U.S.A., Mr. J. A. LePrince, U.S.A., and Maj. Gen. Sir Gordon Covell, India.

SEATED: Gen. Marcel Vaucel, France, Dr. Wilbur A. Sawyer, Secretary General of the Congresses, Dr. L. A. Scheele, Surgeon General, U. S. Public Health Service, and Dr. L. Van Hoof, Belgium.

STANDING: Prof. Alberto Missiroli, Italy, Prof. J. Rodhain, Belgium, Maj. General Sir Gordon Covell, India, Sir. Malcolm Watson, England, Sir Sahib Singh Sokhey, India and Dr. Arnoldo Gabaldon.

WALTER REED EXERCISES—4TH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA

Left to right: Major General M. W. Ireland, Brigadier General J. Kean, Major General W. Lawrence Reed, Major General Raymond W. Bliss, Dr. Fred L. Soper, Program Chairman, Dr. Philip Hench, Mayo Clinic, the Speaker, Mr. J. L. Hanberry, one of Walter Reed's volunteers and Brigadier General Albert A. Truby.

There was widespread sympathy in his last illness. Messages from every part of the world poured in. For at last the world realized something of the high moral purpose of the man and his life. At the memorial service, where governments, universities, learned societies were represented, as well as a great general congregation, his own hymn of praise, written soon after his discovery, was sung:

"Before Thy feet I fall,
Lord, who made high my fate,
For in the mighty small
Thou showest the mighty great."

And now let me close with what has been described by a poet as one of the loveliest things ever written in English, Ross's poem:

The Star

"Far across the Loneland, far across the Sea,
Far across the Sands, O silver shining
Sister of the Silence, Sister of the Dew,
Sister of the Twilight, lighten me.

Ever art thou beaming, I with eyes upcast,
Gazing worn and weary from this Dark World,
Ask of thee thy Wisdom, steadfast Eye of God,
That I be as Thou art while I last."

A PIROPLASM, *BABESIA WRIGHTI*, N. SP., FROM THE ROCK SQUIRREL (*CITELLUS VARIEGATUS BUCKLEYI*)

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(Received for publication 10 September 1948)

Following repeated failures to infect any of the small laboratory mammals with several of the recognized species of malaria, attention was turned to the rodents of the Southwestern United States in the hope that a new species of plasmodia might be found which would better lend itself to this purpose. Through arrangements made with the U. S. Public Health Service Laboratory in San Francisco, one of the authors accompanied a plague survey team from that station for two months during its operation in Southwest Texas and made blood films on all animals that were killed or captured. Examination of several thick blood films from this group revealed an organism so suggestive of plasmodia that the malaria survey work was temporarily interrupted and limited trapping operations were begun in the area from which positive slides had been obtained. This report deals with the studies made on thirteen rock squirrels captured during this operation.

On initial examination a protozoan was seen within the red blood cells of three of these animals. As observed in the first blood films the parasites, especially the smaller ring forms, bore a striking resemblance to *Plasmodium falciparum*. However, by the end of sixty hours, typical piriform bodies (singularly and in groups of two to six) appeared in some blood films. This, together with the absence of pigment in all parasites thus far observed, made it evident that this protozoan was not a plasmodium.

THE PARASITE

As studied in thin blood films stained with Giemsa, the parasites, for convenience, may be divided into three groups.

The *ring forms* measure 1.6 to 4.3 micra in diameter with the smaller ones showing a single, small, dense, darkly staining nucleus and fine, delicate, light blue cytoplasm. Vacuoles may or may not be present in this early stage. As the ring becomes larger the chromatin mass increases in size and usually stains a brighter red while the cytoplasm surrounding the central vacuole becomes more dense. Frequently a signet ring type of parasite is produced which resembles those seen in *P. falciparum* infections. Double chromatin dots are seen in some, while in others the chromatin forms a band stretching across the parasite.

The *ameboid forms*, measuring from 2.7 to 10.7 micra in their greatest diameter, show marked variation in shape. They vary from medium-sized, irregularly oval bodies with one or more short, broad-based pseudopodia to complex protoplasmic masses with thin, intertwining cytoplasmic processes often forming delicate loops. The chromatin often appears as two or more widely separated masses; occasionally

it exists as a single, round, compact nucleus; less frequently it is drawn out into an irregular band or into a chain of coarse granules. In the young ameboid forms the cytoplasm shows usually diffuse, occasionally patchy, peripheral condensation, while in the older forms it is usually drawn out into fine, delicate pale blue threads surrounding one or more vacuoles. An occasional parasite may be laterally compressed into a band extending across the red blood cell.

The *piriform bodies* measure 1 to 2.7 micra in length. The compact, deeply staining nucleus is usually round or oval and situated in the bulbous end, although occasionally it may extend from the expanded end down the side of the parasite as an elongated, marginal mass. Occasionally a second smaller chromatin mass is seen, usually located in the constricted end. The cytoplasm shows focal rarefaction and usually contains a vacuole. From one to six, usually four, piriform bodies are seen within apparently unaltered red blood cells. Their arrangement within the cell does not appear to follow any definite pattern. Occasionally they occur in the form of a fan, rarely as pairs with the pointed extremities joined or closely approximated; and on three occasions it was observed that the four daughter cells had remained attached to one another in the form of a cross.

There appears to be no relation between the intensity of the parasitemia and the predominance of any particular form. Although in heavy infections a moderate transitory anemia is produced with occasional to moderate numbers of immature red blood cells of varying size frequently appearing in the peripheral blood, the organism produces no appreciable alterations in the individual parasitized cell. Diligent search revealed no pigment in any parasite although several hundred organisms were studied under polarized light, and films of heavily parasitized blood stained with flourescent stains were examined under ultraviolet light.

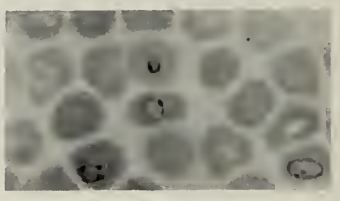
This parasite, which inhabits the red blood cells of the rock squirrel, which exhibits the morphological characteristics described above, and which does not form pigment (hematin), must be considered as belonging to the suborder *Piroplasmidea*. Since the discovery of the first piroplasm by Babes (1888) many new species have been described from a large variety of animals and considerable confusion has existed regarding the nomenclature of the piroplasmata. Accepting the classification of Wenyon (1926) in which the suborder *Piroplasmidea* consits of two families each containing one genus, this parasite would unquestionably belong in the genus *Babesia* since it reproduces asexually within the red blood cells and has not been observed in the fixed tissue cells.

Comparison of this piroplasm with some of the previously described species is difficult, in that drawings, photographs or measurements are lacking in some of the descriptions. However, none of the descriptions, which we have seen, appears to fit adequately this parasite described above. Even the organism described by Macfie (1915) from the brown rat of West Africa (which it most closely resembles) may be differentiated on the basis of the following characteristics presented by Macfie's parasite: vacuoles are always present in even the smallest rings, the nucleus of the piriform body is usually situated near the middle, no paired piriform bodies are found, and the piriform bodies as pictured appear to be larger. This piroplasm from the rock squirrel is therefore proposed as a new species to be designated *Babesia*

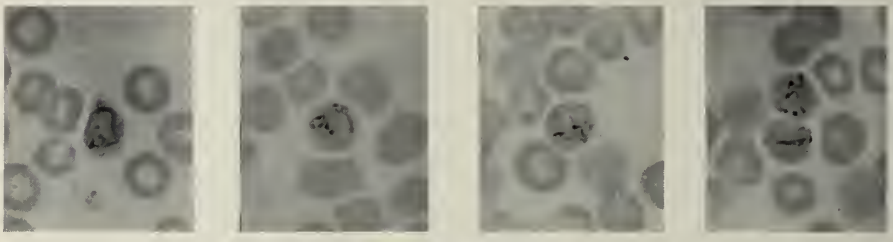
wrighti in honor of Dr. Willard H. Wright, Chief, Division of Tropical Diseases, National Institutes of Health, whose enthusiastic support we have enjoyed throughout this and related projects.

VERTEBRATE HOST

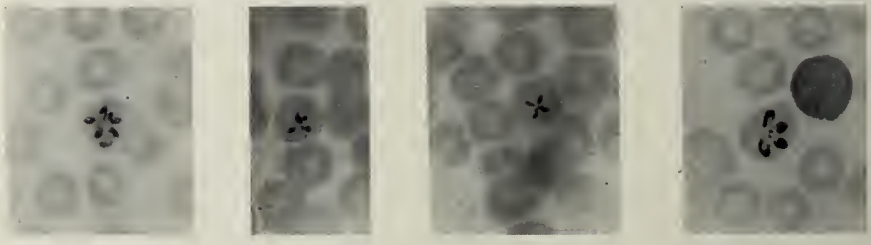
All thirteen animals used in this study were black-backed rock squirrels (*Citellus variegatus buckleyi*) captured three miles east of Junction in Kimble County, Texas,



Ring Forms



Ameboid Forms



Piriform Bodies

FIG. 1. VARIOUS FORMS OF *Babesia wrighti*, N.SP. FROM THE ROCK SQUIRREL (× 900)

during May and June of 1948. Identification was confirmed by Dr. Henry W. Setzer of the United States National Museum.

CLINICAL COURSE

Babesia wrighti was transmitted to five clean animals of the same species by the intramuscular injection of whole citrated blood.

Four of these squirrels were young adults weighing from 475 to 680 grams. In these the parasites appeared in sufficient numbers to be identified from thin blood films on the third and fourth day after inoculation. In animals Nos. 1, 2 and 3, which received over one million parasites each, the highest parasite count (ranging

from 120 to 1720 parasites per 10,000 red blood cells) was observed on the fifth to the seventh day. Squirrel No. 3, which received ten million parasites and showed the highest parasitemia (1720 parasites per 10,000 red blood cells on the sixth day), in addition showed a definite secondary peak of 54 organisms per 10,000 red blood cells on the 25th day. In squirrel No. 4, which received one-half million parasites, the peak parasitemia, which was only 50 parasites per 10,000 red blood cells, was delayed until the twelfth day after inoculation. Usually by the end of the third week after inoculation parasites could be found on thick blood films only.

In the fifth animal a well established infection was not obtained. This was an old animal which weighed 960 grams and which received only 22,500 parasites; babesia were observed only in thick films made on the third through the sixth day after inoculation and were not found during the subsequent two months' observation. In

TABLE 1

Summary of attempts to transmit Babesia wrighti, found in rock squirrel, to other animals

ANIMAL	ROUTE OF INOCULATION	APPROXIMATE NUMBER OF PARASITES INOCULATED	OBSERVATION PERIOD	RESULTS
			days	
White mouse	Intraperitoneal	12 million	19	Neg.
White rat	Intraperitoneal	22,500	15	Neg.
Cotton rat (*Sigmodon hispidus*)	Intraperitoneal	22,500	15	Neg.
Rice rat (*Oryzomys palustris*)	Intraperitoneal	11,250	15	Neg.
Hamster	Intraperitoneal	9,000	15	Neg.
Rabbit	Subcutaneous	9.2 million	14	Neg.
Guinea Pig	Subcutaneous	7.4 million	14	Neg.
Rio Grande ground squirrel (*Citellus mexicanus Parvidens*)	Intramuscular	550,000	41	Neg.
Chick embryo (14 days old)	Intravenous	175,000	7	Neg.
Dog	Subcutaneous	18.5 million	18	Neg.

view of the relatively small number of parasites injected it is not possible to say whether or not this relative refractoriness or poor response was due to having inoculated an old animal which had become resistant because of a previous infection.

At no time during the course of the infection, even with 10 to 15 per cent of the red blood cells parasitized, did any animal appear ill. They maintained their weight, sometimes even gaining, ate well and when handled exhibited their customary agility. The urine remained clear and there was no evidence of icterus. The general picture here appeared to be that of a firmly established, well balanced host-parasite relationship.

Two animals were killed and autopsied, one 28 days after a blood-induced infection and the other (infected in nature) 20 days after capture. At the time of autopsy the former showed ten parasites per 10,000 red blood cells and the latter one parasite per 100 thick film fields. Gross examination revealed no icterus or other abnormal findings and prolonged search of smears from liver, spleen, lung, kidney, lymph nodes and bone marrow revealed no exoerythrocytic parasites. Sections of heart, lung, liver,

spleen, kidney and brain showed on microscopic examination only moderate congestion of the spleen with a moderate amount of phagocytosed hemosiderin in the splenic pulp and slight to moderate patchy-diffuse proliferation of the reticuloendothelial cells.

INFECTIVITY FOR OTHER ANIMALS

Repeated attempts were made to transmit *Babesia wrighti* to a variety of other animals. From both naturally and experimentally infected squirrels blood was withdrawn into sodium citrate and animals were inoculated as shown in Table 1. A careful search was made for parasites in both thick and thin blood films beginning on the third day after inoculation and containuing for a minimum of two weeks. Results in all cases were negative. As a control, in addition to parasite counts made on the donor animal at the time of bleeding, blood was also inoculated into susceptible animals (*C. variegatus buckleyi*) which had been shown to be negative on previous blood examinations. Parasites were demonstrated in all control animals on the third or fourth day after inoculation.

Although this parasite is noticeably smaller than *Babesia canis*, the fact that canine piroplasmosis occurs in Florida and that there is evidence of its existence in Texas made it desirable to determine whether or not this organism would infect the dog. A male dog about one year of age and weighing 8.7 kilograms, which had been housed in the laboratory and subjected to periodic complete blood examinations for seven months, was given 18.5 million parasites subcutaneously and closely followed for 18 days. Throughout this period blood films remained negative for parasites, no elevation of temperature or hemoglobinurea was observed and the blood picture showed no changes.

SUMMARY

1. A non-pigment producing protozoan inhabiting the red blood cells of the black-backed rock squirrel (*Citellus variegatus buckleyi*) is described.

2. It is identified as a member of the genus *Babesia* and is proposed as a new species to be designated *Babesia wrighti*.

3. Failure was experienced in attempting to transmit the infection by blood inoculation to the white mouse, white rat, cotton rat, rice rat, hamster, rabbit, guinea pig, dog, chick embryo and Rio Grande ground squirrel (*Citellus mexicanus parvidens*).

ACKNOWLEDGMENTS

We are indebted to Dr. C. R. Eskey, Medical Officer in Charge, and to Mr. John S. Adams, Survey Aide, U. S. Public Health Service Laboratory, San Francisco, California for assistance in helping to make available the material on which the original blood survey was made, and to Miss Aimee Wilcox for assisting in the interpretation of some of the findings here recorded.

REFERENCES

Babes, V. 1888. Sur l'hemoglobinurie bacterienne du boeuf. C. R. Acad. Sci., **107**: 692.

Macfie, J. W. S. 1915. Babesiasis and Trypanosomiasis at Accra, Gold Coast, West Africa. Ann. Trop. Med. & Parasit., **9**: 457.

Wenyon, C. M. 1926. Protozoology, William Wood and Co., New York.

ALTERATIONS IN SOME CONSTITUENTS OF THE MONKEY ERYTHROCYTE INFECTED WITH *PLASMODIUM KNOWLESI* AS RELATED TO PIGMENT FORMATION[1]

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That profound changes in the host erythrocyte occur upon the entry and subsequent growth of the malaria parasite is well known from microscopic studies. When the erythrocytes of the *Macaca mulatta* monkey are invaded by *Plasmodium knowlesi* the most pronounced changes occur in the last half of the one-day (quotidian) cycle and are associated with a rapid formation of parasite pigment (hematin) and a marked reduction in the hemoglobin of the host cell. In beginning this study we made the tentative assumption that the parasite pigment was formed quantitatively from the hemoglobin of the host erythrocyte and that the total hematin of the infected erythrocyte remained constant. Ball and associates (1948) have recently published experimental data which prove the above assumption to be correct. Accordingly, it is possible to measure the total erythrocyte concentration of a given sample of blood or cell suspension by a determination of the total hematin. This is valid for normal or parasitized cells. It is also possible to measure the average effective number of pigmented parasites of average age in a cell suspension by a determination of parasite hematin. The alterations in the total nitrogen, the total lipides and the total solids of infected erythrocytes have been measured in relation to the amount of parasite pigment.

We have made a preliminary report (Morrison and Jeskey, 1947) on part of the material covered in this paper and on the composition of the isolated parasites.

MATERIALS AND METHODS

Oxalated or citrated blood from normal *Macaca mulatta* monkeys infected with *Plasmodium knowlesi* was centrifuged to remove the plasma. The cells were then washed four times with approximately twenty volumes of 0.9 per cent NaCl solution and then suspended in the same solution to make a cell volume of from 10 to 15 per cent. Aliquots of these cell suspensions were analyzed for total hematin, hemoglobin, total nitrogen, total lipides and lipide-free solids.

In one group of experiments total nitrogen, total hematin and hemoglobin were determined on similarly washed erythrocytes of 16 normal ducks and 15 ducks infected with *P. lophurae*.

Hemoglobin was determined spectrophotometrically by the cyanomethemoglobin

[1] This study was aided by a grant from the Tennessee Valley Authority through the Department of Preventive Medicine of the University of Tennessee.

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method of Drabkin and Austin (1935) after removal of the parasites by centrifugation. Total hematin was determined by the acid acetone method previously employed (Morrison and Anderson, 1941) with the readings at 540 mμ instead of at 640 mμ. All readings were made on a Bausch and Lomb universal spectrophotometer.

A semi-micro kjeldahl method was used for total nitrogen using a digestion mixture of copper sulfate, potassium sulfate and sulfuric acid, the ammonia being distilled into boric acid and titrated with standard acid to a matched endpoint using methyl red as an indicator.

The iodometric method of Bloor (1928) was used for the determination of total lipides. Lipide-free solids were determined gravimetrically on the residues from the lipide extractions. All determinations were made in duplicate or in triplicate.

TABLE 1

Constituents of Normal Monkey Erythrocytes

HEMOGLOBIN mM* PER LITER	TOTAL HEMATIN mM PER LITER	TOTAL NITROGEN GM. PER MM OF HEMATIN	TOTAL LIPIDES MG. PER mM OF HEMATIN	SOLIDS GM. per mM OF HEMATIN
3.17	3.17	2.85	249	18.36
3.59	3.56	2.91	269	18.90
3.47	3.51	2.92	255	18.97
3.57	3.61	2.90	265	18.30
2.70	2.83	2.90	257	18.69
Ave..3.30.....	3.34	2.90	259	18.64
Standard deviation.....		0.025	7.16	0.27
Coefficient variation per cent.....		0.86	2.75	1.45

* Milli-mol.

RESULTS

In Table 1 are presented the data on the constituents of the erythrocytes of five normal monkeys. From the data presented, one may evaluate the reproducibility of the methods employed. In addition we have previously determined the ratio of total nitrogen to total hematin on the washed erythrocytes of six normal monkeys and found 2.96 gm. of nitrogen to 1 mM of hematin with a coefficient of variation per cent of 3.55. From these data and the data in Table 1 it may be calculated that there are from 207 to 212 atoms of nitrogen per mol. of hematin.

We have attempted to determine whether the conversion of the hemoglobin to parasite pigment was quantitative and whether the total hematin of the cell remained constant. It appeared possible that the nucleated avian erythrocyte might be more resistant to the ravages of *Plasmodium* infection than the non-nucleated monkey erythrocyte. The washed erythrocytes of 16 normal ducks were found to contain 256 atoms of nitrogen (C. V. per cent of 2.34) to 1 mol. of hematin while the cells of 15 ducks infected with *P. lophurae* were 247 atoms. (C. V. per cent of 1.54) In this series from 4 to 38 per cent of the total pigment was in the form of parasite hematin with an average of 22 per cent for the group. While this difference is only 3.5 per

cent it is statistically significant. Recently Ball et al (1948) have demonstrated by *in vitro* studies of the metabolism of *P. knowlesi* that the conversion of hemoglobin hematin to parasite hematin is quantitative and that the total hematin of the cell remains constant.

From our data the nucleated duck erythrocyte has approximately 22 per cent more nitrogen per mol. of hematin than the non-nucleated monkey erythrocyte. Further, there is relatively little loss of nitrogen resulting from the parasite metabolism.

In figure 1 are presented the data on the relation of the per cent of parasite pigment formed to the per cent of cells infected by all forms of the parasite and to the per cent of cells infected by only the pigmented forms. It is apparent that there is no quantitative relation when all forms are considered but that there is for the pig-

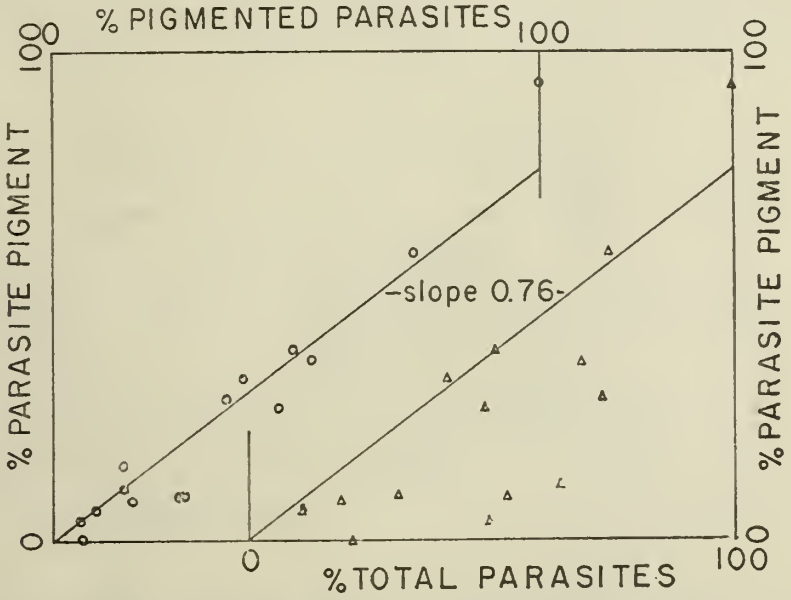

FIG. 1. RELATION OF PARASITE PIGMENT TO THE PER CENT OF PARASITIZED CELLS

mented forms. The calculated line slope of 0.76 indicates that 0.76 per cent of the hemoglobin has been converted to parasite hematin when 1.0 per cent of the cells are infected with the average pigmented form of parasite. Obviously, this value could vary with the relative distribution of the pigmented forms as to late ameboid, pre-segmenter or segmenter and to the number of cells with multiple infections.

In order to concentrate the more mature parasites we have resorted to fractional centrifugation and the circle at 74 per cent parasites and 59 per cent pigment, figure 1, was obtained on cells concentrated in this way. To obtain the cells with 100 per cent infection with pigmented forms the entire blood obtained from one animal in which the cells contained 35 per cent of pigmented forms was repeatedly fractionated to obtain a final suspension in which all the parasites were heavily pigmented.

Ball et al (1948) present data on the blood of two monkeys infected with *P. knowlesi*. On one they found 40 per cent of the total hematin as free hematin with

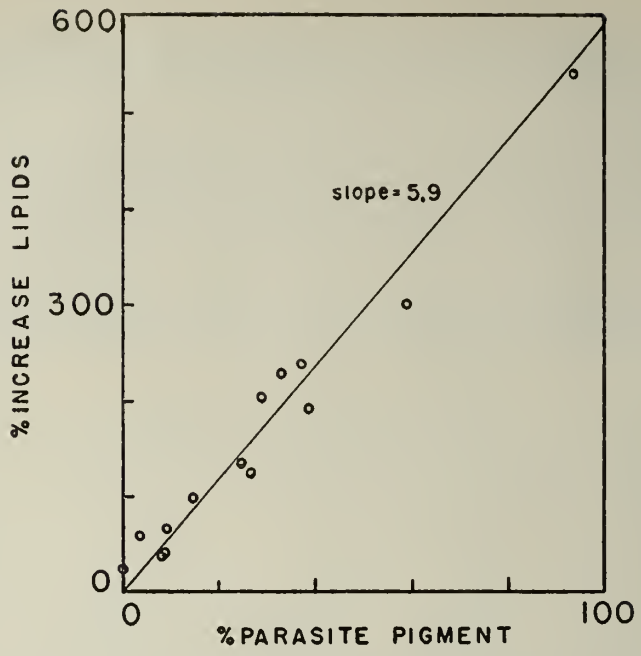

FIG. 2. INCREASE IN THE TOTAL LIPIDES OF THE CELL RELATED TO THE PER CENT PARASITE PIGMENT

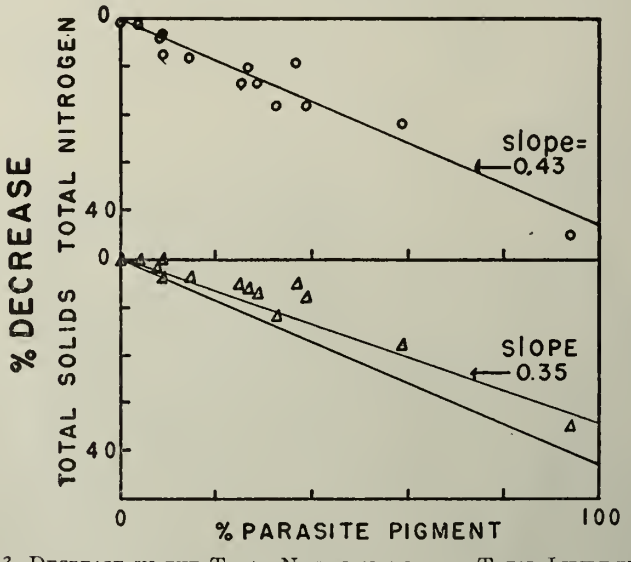

FIG. 3. DECREASE IN THE TOTAL NITROGEN AND THE TOTAL LIPIDE-FREE SOLIDS OF THE CELL RELATED TO TOTAL PARASITE PIGMENT

56 per cent of the cells infected with pigmented forms and a second with 49 per cent free hematin and 62 per cent of pigmented forms. From these data we calculate that

0.72 and 0.79 per cent, respectively, of the hemoglobin has been metabolized for a 1.0 per cent parasitemia as compared with our average of 0.76 per cent.

The data in figure 2 indicate that there is an average increase of 5.9 per cent in total lipides for an increase of 1.0 per cent in parasite pigment. It is to be expected from our findings (Morrison and Jeskey, 1947) that the isolated dried parasites contain 28.8 per cent total lipides. Ball et al (1948) have reported an increase of 400 per cent in total fatty acids.

The marked reduction in total nitrogen and in total lipide-free solids is apparent from the data in figure 3. The calculated slope 0.43 of the line (upper panel) indicates a loss of 0.43 per cent of total nitrogen for 1.0 per cent increase in parasite pigment. This loss is total nitrogen appears to result from the metabolism of the globin of the host cell hemoglobin and is partially compensated for by the protein of the parasite. The parasite protein which makes up 60.7 per cent of the dried parasite is only approximately 14 per cent nitrogen (Morrison and Jeskey, 1947).

TABLE 2

Summary of the Changes in Erythrocyte Constituents

	(A)	(B)	(C)
Hemoglobin mM	1	0	0.24
Parasite hematin mM	0	1	0.76
Total nitrogen, gm.	2.90	1.65	2.18
Total lipides, gm.	0.259	1.787	1.358
Lipide free solids, gm.	18.64	12.12	15.95
Total solids, gm.	18.90	13.91	17.31

(A) Experimental values for normal erythrocytes per 1 milli-mol of total hematin.

(B) Calculated values for infected erythrocytes with 100 per cent parasitemia and 100 per cent pigment.

(C) Calculated values for the erythrocytes 100 per cent infected with average pigmented forms and 76 per cent pigment.

Lipide-free solids decrease 0.35 per cent for 1.0 per cent increase in parasite pigment as shown in the lower panel of figure 3. Since there appears to be no marked alteration in the size of the host cell as a result of parasitemia by this particular *plasmodium* it would appear that there is a net loss of solids per unit of cell volume. Moulder and Evans (1946) have demonstrated an active amino acid metabolism in chicken erythrocytes infected with *P. gallinaceum.*

DISCUSSION

A summary of the experimental data and of certain calculations from these data is presented in Table 2. The values given are all calculated to a quantity of erythrocytes containing 1 mM of hematin. This quantity of normal erythrocytes suspended in or 0.9 per cent NaCl to make a final volume of 100 ml would contain 16.47 gm of hemoglobin with an oxygen capacity of 22.4 ml and is within the range found in normal monkey blood.

In column A are found the average values for the cells of 5 normal monkeys. The

data in column B are the values calculated for a complete quantitative conversion of the hemoglobin hematin to parasite hematin and taken from data in Table 1 and figures 2 and 3. Total solids in all cases are obtained by adding the values for the lipide-free solids and total lipides.

Since it is highly improbable that the hemoglobin is completely metabolized in a cell even with a mature multiple infection we can use these data to calculate the changes in the constituents of the erythrocytes when completely infected with the average pigmented forms of *P. knowlesi.* The values obtained from such calculations are shown in column C and are based on the data, figure 1 that when all the cells are infected with the average pigmented forms of the parasite that 76 per cent of the hemoglobin will have been converted to parasite pigment. There is a reduction of 24.8 per cent in total nitrogen; 14.5 per cent in lipide-free solids; 8.4 per cent in total solids but a 423 per cent increase in total lipides. The reduction in total solids is not as great as the reduction in lipide-free solids due to the relatively large increase in total lipides. The total lipides account for 7.85 per cent of the total solids. This increase in total lipides and decrease in total solids would account for the specific gravity of 1.07 reported by Ferrebee and Geiman (1946) for the parasitized erythrocytes.

SUMMARY

Alterations in some of the constituents of the monkey erythrocyte as a result of *Plasmodium knowlesi* metabolism have been studied quantitatively. The host cell hemoglobin is 76 per cent converted to parasite pigment as a result of the metabolism of an average pigmented form of parasite. This metabolism also decreases the total nitrogen and total solids of the cell but increases the total lipides. In such a cell the lipides account for 7.85 per cent of the total solids.

BIBLIOGRAPHY

Ball, E. G., McKee, R. W., Anfinson, C. B., Cruz, W. O., and Geiman, Q. M. (1948) Studies on malarial parasites. IX. Chemical and metabolic changes during growth and multiplication in vivo and vitro. J. Biol. Chem., **175:** 547–571.

Bloor, W. R. (1928) Determination of lipids in plasma. J. Biol. Chem., **77:** 53–73.

Ferrebee, J. W., and Geiman, Q. M. (1946) Studies on malarial parasites. III. A procedure for preparing concentrates of Plasmodium vivax. J. Infect. Dis., **78:** 173–179.

Moulder, J. W., and Evans, E. A., Jr. (1946) The biochemistry of the malaria parasite. VI. Studies on the nitrogen metabolism of the malaria parasite. J. Biol. Chem., **164:** 145–157.

Drabkin, D. L., and Austin, J. H. (1935) Spectrophotometric studies. II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. J. Biol. Chem., **112:** 51–65.

Morrison, D. B., and Anderson, W. A. D. (1942) On the role of parasite pigment in the malaria paroxysm. Public Health Reports, **57:** 161–175.

Morrison, D. B., and Jeskey, H. A. (1947) The pigment, lipids, and proteins of the malaria parasite (Plasmodium knowlesi). Federation Proc., **6:** 279.

HYPERSENSITIVITY IN SIMIAN MALARIA[1]

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While the role of the humoral antibodies in malaria has been extensively investigated little work has been done on the problem of hypersensitivity in this disease. Herrmann and Lifschitz (1930), using watery extracts of blood clots from malarial patients, described a delayed cutaneous reaction in patients with acute and chronic malaria. Sinton and Mulligan (1932), after failing to obtain cutaneous reactions in malarial monkeys by inoculating the washed unaltered mass of the bodies of *Plasmodium knowlesi*, used a papain digest of the malarial parasites as antigen. Intracutaneous inoculations of the latter produced an immediate reaction in both normal and infected monkeys; but at 24 hours the reactions had faded away in the normal monkeys and reached a maximum in the infected animals. Dulaney and Stratman-Thomas (1940), working with the methods of Sinton and Mulligan as well as those of Herrmann and Lifschitz, were unable to demonstrate a specific cutaneous reaction in human malaria. Taliaferro and Bloom (1945) noted that monkeys infected with *P. knowlesi* reacted to cutaneous injections of malarial blood by agglutinating erythrocytes containing *P. knowlesi*. Microscopically, the inflammation was more pronounced in the skin of the immune monkeys than in the skin of the normal monkeys. The gross appearance of these reactions in the normal and immune monkeys were not described, however. Recently Makari (1946) described a high incidence of positive reactors in patients with acute and chronic malaria following an intradermal injection of an antigen prepared from whole blood of chickens infected with *P. gallinaceum*.

In the present experiments studies were made of the cutaneous reactions of rhesus monkeys, both normal and malarial, to antigens prepared from *P. knowlesi* and *P. gallinaceum*. The use of enzyme-extracts of the parasite mass was deliberately avoided because the enzyme itself would prove irritating to the tissues. An attempt was also made to enhance sensitization artificially in a few animals by the use of adjuvants. In addition, macrophages from infected and normal monkeys were tested for sensitivity to malarial antigen in hanging-drop preparations.

METHODS AND MATERIALS

Animals

All the monkeys used in the tests were of the species *Macacca mulatta*. Of these, 26 were normal, 5 had been infected with *P. cynomolgi* and 16 with *P. knowlesi*. The monkeys infected with *P. knowlesi* were treated with sulfadiazine in order to produce chronic infections.

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Antigens

P. knowlesi antigen I. The parasitized blood of monkeys infected with *P. knowlesi* was drawn off with sodium citrate, and the erythrocytes hemolyzed with distilled water. The parasites were washed repeatedly with distilled water, dried in a vacuum, and ground to a fine powder (Dulaney and Stratman-Thomas, 1940). A suspension of the powdered antigen was made in distilled water in a concentration of 1 per cent by weight.

P. knowlesi antigen II. An extract was made of the dried parasites by adding 100 parts of distilled water to 1 part of the dried parasites by weight. This was kept at room temperature for one hour; the supernatant was used as the antigen after settling of the larger particles by gravity had taken place. This extract was essentially equivalent to the antigen used by Dulaney and Stratman-Thomas (1940) for the complement-fixation test for malaria.

P. knowlesi antigen III. An extract of 1 in 100 in distilled water was made from the dried parasites by keeping the mixture in the refrigerator for 24 hours. The supernatant fluid was used after the larger particles had settled out by gravity.

P. knowlesi antigen IV. A 1 in 40 extract of the dried parasites in Tyrode's solution was made by repeated freezing and thawing (10 times) with dry ice and alcohol. The supernatant was used after the larger particles had settled out.

P. knowlesi antigen V. The wet mass of malarial parasites was dissolved in 0.1 N NaOH, using 25 cc. to 1 gram of parasites. This was left overnight at room temperature; by this time the parasites were completely dissolved. The solution was neutralized with dilute phosphoric acid to bring the pH to about 8.0. This gave a final concentration of about 2.7 per cent of the wet parasite mass, with a final concentration of about 1.62 per cent protein, 0.81 per cent lipid and 0.27 per cent pigment (Dulaney and Morrison, 1944).

Control antigen from normal monkey blood. This consisted of a 1 in 100 extract in distilled water of hemolyzed erythrocytes from normal monkeys.

P. gallinaceum antigen I. The material used was "Malarial Antigen (Dried)" prepared by Lederle Laboratories, Inc. at Pearl River, N. Y. for the Army Medical School for use in the serodiagnosis of malaria by the complement-fixation test. A 1 in 100 suspension of the powdered antigen was made in distilled water.

P. gallinaceum antigen II. A 1 in 100 extract in distilled water was made from the dried antigen by keeping the suspension at room temperature for one hour. The supernatant fluid was used after the larger particles had settled out.

Control Antigen from normal chicken blood. This consisted of a 1 in 100 suspension in distilled water of the dried material obtained from hemolyzed blood of normal chickens.

Inoculations. In all tests 0.1 cc. of the test material was inoculated intracutaneously. The extracts were made up in small quantities and kept frozen until ready for use. The suspensions of larger particles (*P. knowlesi* antigen I and *P. gallinaceum* antigen I) were made up fresh each time. Care was taken to keep dosages uniform by shaking the suspensions immediately before each inoculation. Readings were made at $\frac{1}{2}$, 24, 48 and 72 hours after the inoculations. The maximum responses to all antigens occurred between 24 to 48 hours, usually at 24 hours.

RESULTS

P. knowlesi antigen I. The results obtained with this antigen are shown in Table 1. Twenty-six normal monkeys were treated with this antigen. The reactions in this group were always 5 mm. in diameter or less, with a mean diameter of 3.7 mm.

Tests were done on 5 monkeys which had been infected with *P. cynomolgi.* One monkey, which had been infected 6 weeks prior to the test, showed a reaction of 8 mm. in diameter; two animals which had been infected three months previously showed reactions 7 mm. in diameter, and two which had been infected 5 months

TABLE 1

Cutaneous reactions of normal and malarial monkeys to intracutaneous inoculations of P. knowlesi *antigen I*

DIAMETER OF REACTION	NUMBER OF ANIMALS						
	Normal	*P. cynomolgi* infection 1.5 to 5 months	*P. knowlesi* infection—weeks				
			1 to 2	2 to 4	4 to 10	10 to 20	20 or more
mm.							
0	1	0	0	0	0	0	2
1	0	0	0	0	0	0	0
2	3	0	0	0	0	0	1
3	4	0	2	0	0	0	0
4	12	0	1	0	0	0	1
5	6	2	1	5	2	5	3
6	0	0	1	3	4	5	0
7	0	2	2	0	1	0	0
8	0	1	0	0	0	2	0
Mean diameter in mm.....	3.69	6.40	5.00	5.38	5.86	5.92	3.00
Standard error of the mean	0.234	0.600	0.655	0.183	0.261	0.313	0.873
t (on comparison with normal).....		4.555	2.348	3.842	5.485	5.482	
p.....		$<.001$	$<.05 > .01$	$<.001$	$<.001$	$<.001$	

previously gave reactions 5 mm. in diameter. The mean diameter was 6.4 mm., greater than that of the normal group by 2.7 mm. This difference in the means was found to be highly significant, since p was less than .001.

The remaining tests were done on monkeys infected with *P. knowlesi.* Seven monkeys were tested within 1 to 2 weeks after the onset of infection. The mean diameter in this group was 5.0 mm., slightly greater than that of the normal group. This difference was significant, since p fell between .05 and .01. Eight monkeys were tested 2 to 4 weeks after infection. The mean diameter in this group was 5.38 mm., and the difference from the mean of the normal group was highly significant. In the 4 to 10 week period after infection tests were done on seven monkeys, resulting in a mean diameter of 5.86 mm. In the 10 to 20 week period 12 monkeys were tested,

with a resulting mean diameter of 5.92 mm. In the latter two groups the differences from the mean of the normal group were both highly significant. After 20 weeks the increased cutaneous reactivity apparently disappeared because in this group the results of tests on seven monkeys gave a mean diameter of 3.0 mm.

P. knowlesi antigen II. The reactions of the malarial monkeys to this antigen were no greater than those of the normal monkeys (Table 2).

P. knowlesi antigen III. The mean diameter of the reactions of seven normal monkeys to this antigen was 2.29 mm. Five monkeys tested within 4 to 8 weeks after infection with *P. knowlesi* showed a mean diameter of 5.2 mm. The difference between these two means was statistically significant. After 8 weeks, however, the

TABLE 2

Cutaneous reactions of normal and malarial monkeys to intracutaneous inoculations of P. knowlesi *antigen II*

DIAMETER OF REACTION	NUMBER OF ANIMALS		
	Normal	*P. knowlesi* infection—weeks	
		4 to 9	9 to 20
mm			
0	1	0	0
1	0	0	3
2	0	0	6
3	2	1	3
4	1	0	1
5	3	3	0
6	0	0	0
7	0	1	0
Mean diameter in mm.....	3.57	5.00	2.15
Standard error of the mean...	0.685	0.632	0.249
t (on comparison with normal).....		1.53	
p.....		$<.5>.1$	

reactions of the malarial monkeys approximated those of the normal animals (Table 3).

P. knowlesi antigen IV. In this group also there was a small but significant difference in the mean diameters of the reactions of the normal group and those of the monkeys tested during the 4 to 8 week period after infection with *P. knowlesi.* After 8 weeks however the increased reactivity to this antigen disappeared. (Table 4).

P. knowlesi antigen V. No difference was seen in the reactions of the malarial and normal monkeys to this antigen (Table 5).

Control antigen from normal monkey blood. The great majority of normal and malarial monkeys showed no reactions to this antigen; in a few animals however this material produced a response of about 3 mm. in diameter.

TABLE 3

Cutaneous reactions of normal and malarial monkeys to intracutaneous inoculations of P. knowlesi *antigen III*

DIAMETER OF REACTION	NUMBER OF ANIMALS		
	Normal	*P. knowlesi* infection—weeks	
		4 to 8	8 to 20
mm.			
0	2	0	2
1	1	0	1
2	1	1	6
3	0	0	3
4	2	0	1
5	1	1	0
6	0	2	0
7	0	1	0
Mean diameter in mm.....	2.29	5.20	2.00
Standard error of the mean...	0.778	0.860	0.320
t (on comparison with normal).....		2.509	
p.....		$<.05>.01$	

TABLE 4

Cutaneous reactions of normal and malarial monkeys to intracutaneous inoculations of P. knowlesi *antigen IV*

DIAMETER OF REACTION	NUMBER OF ANIMALS		
	Normal	*P. knowlesi* infection—weeks	
		4 to 8	8 to 20
mm.			
0	1	0	1
1	2	0	2
2	1	0	5
3	1	0	1
4	2	1	2
5	3	4	0
Mean diameter in mm.....	3.00	4.80	2.09
Standard error of the mean...	0.596	0.179	0.368
t (on comparison with normal).....		2.892	
p.....		$<.05>.01$	

P. gallinaceum antigen I. This antigen was tested on 12 normal monkeys and produced reactions of 5 mm. or less, with a mean diameter of 3.08 mm. The five monkeys which had been infected with *P. cynomolgi* gave reactions less than 5 mm. with a mean diameter of 2.4 mm. In the case of monkeys infected with *P. knowlesi* the mean diameter of the reactions was 3.5 mm. at 2 to 4 weeks (no greater than the normal), 7.0 mm. at 4 to 9 weeks (significantly greater than the normal), and 2.7 mm. at 9 to 20 weeks. (Table 6).

P. gallinaceum antigen II. The mean diameter in the normal group was 1.92 mm. That of the group infected with *P. cynomolgi* was 1.6 mm. In the case of the monkeys infected with *P. knowlesi* the only significant reactions occurred during the 4 to 9 week period after the onset of the infections. In this period the mean diameter

TABLE 5

Cutaneous reactions of normal and malarial monkeys to intracutaneous inoculations of P. knowlesi *antigen V*

DIAMETER OF REACTION	NUMBER OF ANIMALS		
	Norma	*P. knowlesi* infection—weeks	
		4 to 9	12 to 20
mm.			
0	0	0	2
1	0	1	0
2	3	1	1
3	5	1	0
4	3	1	0
5	1	2	2
Mean diameter in mm.....	3.17	3.33	2.40
Standard error of the mean...	0.272	0.667	1.124
t (on comparison with normal).....		0.222	
p.....		$<.9>.5$	

was 4.4 mm., and the difference of this mean from that of the normal group was significant, p having an approximate value of .01.

Control antigen from normal chicken blood. The normal and malarial monkeys reacted equally to this material. The normal group showed a mean diameter of 4.0 mm., the monkeys infected with *P. cynomolgi* a mean diameter of 5.0 mm., and the animals infected with *P. knowlesi* a mean diameter of 3.6 mm.

Attempt to enhance sensitization by adjuvants. An attempt was made to enhance sensitization by means of inoculating antigen together with adjuvants (Freund and Walter, 1944; Freund, Thomson, Sommer, Walter and Schenkein, 1945). Three monkeys were chosen that had been incoulated with *P. knowlesi* three months earlier. Each monkey was given a subcutaneous inoculation of 1.8 cc. of the following mix-

TABLE 6

Cutaneous reactions of normal and malarial monkeys to intracutaneous inoculations of P. gallinaceum *antigen I*

DIAMETER OF REACTION	NUMBER OF ANIMALS				
	Normal	*P. cynomolgi* infection 9 to 25 weeks	*P. knowlesi* infection—weeks		
			2 to 4	4 to 9	9 to 20
mm.					
0	1	1	0	0	0
1	0	1	0	0	2
2	2	0	0	0	4
3	5	2	4	0	3
4	2	0	1	0	2
5	2	1	1	1	1
6	0	0	0	1	0
7	0	0	0	1	0
8	0	0	0	1	0
9	0	0	0	1	0
Mean diameter in mm.....	3.08	2.40	3.50	7.00	2.67
Standard error of the mean..	0.398	0.871	0.341	0.707	0.355
t (on comparison with normal).....				4.831	
p.....				$<.001$	

TABLE 7

Cutaneous reactions of normal and malarial monkeys to intracutaneous inoculations of P. gallinaceum *antigen II*

DIAMETER OF REACTION	NUMBER OF ANIMALS				
	Normal	*P. cynomolgi* infection 9 to 25 weeks	*P. knowlesi* infection—weeks		
			2 to 4	4 to 9	9 to 20
mm.					
0	3	1	0	0	2
1	2	2	2	0	3
2	1	0	2	0	5
3	5	2	1	2	0
4	1	0	0	1	1
5	0	0	1	1	1
6	0	0	0	0	0
7	0	0	0	1	0
Mean diameter in mm.....	1.92	1.60	2.33	4.40	1.83
Standard error of the mean..	0.417	0.600	0.615	0.748	0.423
t (on comparison with normal).....				2.896	
p.....				Approx. 0.01	

ture: 2 cc. of Falba, 2 cc. of mineral oil containing 10 mgm. of dried tubercle bacilli and 3 cc. of sterile saline containing 30 mgm. of the powdered malarial antigen (*P. knowlesi*). Prior to this treatment the animals gave reactions of 5,6 and 6 mm. in diameter respectively. One month after the inoculation of the adjuvants these animals showed reactions of 5,5 and 8 mm. respectively. Thus there was no increased cutaneous reactivity as a result of the inoculation of the antigen-adjuvant mixture.

Tests for macrophage sensitivity in vitro. Tests were made for macrophage sensitivity in hanging-drop preparations. The macrophages were obtained from the clotted buffy coat of peripheral blood of normal and malarial monkeys. The fragments of buffy coat were embedded in plasma from both normal and malarial monkeys. To the plasma, while still fluid, was added sterile powdered antigen (*P. knowlesi*) in final concentrations of 1 in 10,000 to 1 in 1000. The cultures were incubated at 37 degrees C. for four days and were examined daily. This was done in the case of three normal monkeys and three monkeys which had been infected with *P. knowlesi* three months previously; of the latter, one monkey had been inoculated with the antigen-adjuvant mixture one month previously. No difference was seen in the behavior of the macrophages from the normal and malarial monkeys. It was felt that these concentrations of the antigen were not high enough, but suspensions of the powdered antigen in a concentration of 1 in 100 so obscured the field of the hanging-drop preparations that no conclusions could be reached about the appearance and behavior of the macrophages at this concentration. Accordingly a highly concentrated aqueous extract of the powdered antigen was prepared by repeated freezing and thawing; this was used in a final concentration of 1 in 40 in the case of the normal and malarial monkeys so previously tested. Again no difference was seen in the macrophage cultures.

DISCUSSION

The antigen which gave the best results was the suspension of the powdered bodies of *P. knowlesi*. In the case of the monkeys with *P. knowlesi* infections the cutaneous reactivity showed a small but significant increase over that of the normal group as early as 1 to 2 weeks after infection. This increased to a peak between 4 to 20 weeks, and returned to normal after 20 weeks. Cross reactivity with *P. cynomolgi* was demonstrated by increased cutaneous reactions of monkeys with chronic infections with this parasite.

The failure of *P. knowlesi* antigen II (aqueous extraction for 1 hour) to produce significant reactions probably means that the fractions responsible for the cutaneous responses are not readily soluble in water. That they can be so extracted in weak concentration is shown by the small reactions produced by *P. knowlesi* antigen III (aqueous extraction for 24 hours) and *P. knowlesi* antigen IV (aqueous extraction by repeated freezing and thawing). These reactions, however, were elicited only in the second month after infection. The lack of response to *P. knowlesi* antigen V may be due to denaturation of the antigen by the sodium hydroxide. Dulaney and Morrison (1944) noted that a similar method of preparation of malarial antigen for use in complement-fixation also resulted in a poor antigen.

The two antigens prepared from *P. gallinaceum* were less active. They produced

cutaneous responses only during the 4 to 9 week period after infection with *P. knowlesi*. After 9 weeks the reactions of the malarial monkeys returned to the normal level. Although these reactions were weak they nevertheless demonstrated cross reactivity between *P. knowlesi* and *P. gallinaceum*. The failure of the monkeys infected with *P. cynomolgi* to respond to the *P. gallinaceum* antigens was probably due to the fact that they were tested after 9 weeks of infection, at which time the monkeys infected with *P. knowlesi* also failed to react to these antigens. At the same time that the animals infected with *P. cynomolgi* failed to react to the *P. gallinaceum* antigens they showed significant responses to *P. knowlesi* antigen I.

These results demonstrate that rhesus monkeys infected with *P. knowlesi* or *P. cynomolgi* develop a certain degree of hypersensitivity to malarial antigen which, in terms of response to *P. knowlesi* antigen I, lasts about 5 months. These cutaneous reactions are of the delayed type. The failure to obtain marked cutaneous reactions probably resulted from (1) the weakness of the antigens and (2) the fact that monkeys are difficult animals to sensitize to antigens (Topley and Wilson, 1946).

In the interpretation of the results of this study consideration should be given to the possible sources of error inherent in the methods used. These are: (1) the small size of the reactions and the difficulty of obtaining accurate measurements, (2) the difficulty of obtaining uniform suspensions of the antigens, and (3) the small number of animals in some of the test groups.

A question that arises is whether or not hypersensitivity is of any importance in the pathogenesis of malarial disease. The work of Taliaferro and Bloom (1945) suggests that the increased reactivity may exert a protective effect by producing greater localization and phagocytosis of the parasitized cells. In the present experiments there was no evidence that the hypersensitivity resulted in any harmful effects in the monkeys. On the other hand, in tissue sections of lymph nodes from monkeys suffering from chronic infections with *P. cynomolgi* or *P. knowlesi* I have occasionally seen extreme degrees of erythrophagocytosis involving non-parasitized cells. This suggests that an immune response may be of significance in the destruction of erythrocytes, both parasitized and non-parasitized, with the resultant production of anemia. The *in vitro* studies of Zuckerman (1945) which demonstrated opsonins in the serum from birds immune to malaria also showed that in the presence of the immune serum the macrophages phagocytosed parasitized and non-parasitized cells with equal avidity. Finally, the work of Mayer and Heidelberger (1946) demonstrated that in human malaria the stromata of erythrocytes may act as antigen resulting in the production of complement-fixing antibodies which were distinct from those which fixed complement in the presence of the malarial antigen.

The attempt to enhance sensitization by subcutaneous inoculation of an antigen-adjuvant mixture was unsuccessful.

The tests for macrophage sensitivity to malarial antigen were negative, probably due to the weakness of the antigen. In the experiments of Rich and Lewis (1932) where sensitivity of macrophages to tuberculin was demonstrated, concentrations of tuberculin as high as 1 in 80 to 1 in 60 had to be employed. These concentrations represent at least 12 times the dosage necessary to produce a cutaneous reaction in tuberculous guinea-pigs. In the present experiments the concentration of antigen

used in the skin tests was 1 per cent of the dried powder, while the highest concentration achieved in the macrophage tests was 1 in 40 of an aqueous extract.

SUMMARY AND CONCLUSIONS

1. The cutaneous reactions of monkeys infected with *P. knowlesi* to intracutaneous inoculations of antigen prepared from the dried bodies of *P. knowlesi* were significantly greater than those of normal monkeys.

2. The hypersensitivity of the infected monkeys occurred as early as 1 to 2 weeks after infection, reached a peak between 4 to 20 weeks, and disappeared after 20 weeks.

3. Aqueous extracts of the dried antigen produced weaker antigens which elicited hypersensitive reactions confined to the second month of infection.

4. Monkeys with chronic infections with *P. cynomolgi* also showed small but significant allergic cutaneous reactions to the dried *P. knowlesi* antigen.

5. The animals infected with *P. knowlesi* gave allergic cutaneous reactions to antigens prepared from *P. gallinaceum* but these occurred only during the second month of infection.

6. Cross reactivity was demonstrated between *P. knowlesi* and *P. cynomolgi* as well as between *P. knowlesi* and *P. gallinaceum*.

7. An attempt to enhance sensitization by the use of adjuvants was unsuccessful.

8. Macrophages from both normal and malarial monkeys failed to show any sensitivity to malarial antigen.

REFERENCES

Dulaney, A. D., and Morrison, D. B. 1944. On the Preparation of Antigens from *Plasmodium knowlesi*. Amer. Jour. Trop. Med. **24:** 323–326.

Dulaney, A. D., and Stratman-Thomas, W. K. 1940. Complement Fixation in Human Malaria; Results Obtained with Various Antigens. Jour. Immunol. **39:** 247–255.

Freund, J., and Walter, A. W. 1944. Saprophytic Acidfast Bacilli and Paraffin Oil as Adjuvants in Immunization. Proc. Soc. Exper. Biol. and Med. **56:** 47–50.

Freund, J., Thomson, K. J., Sommer, H. E., Walter, A. W., and Schenkein, E. L. 1945. Immunization of Rhesus Monkeys against Malarial Infection (*P. knowlesi*) with Killed Parasites and Adjuvants. Science **102:** 202–204.

Herrmann, O., and Lifschitz, M. 1930. Intrakutane Reaktion als Diagnose der Malaria. Zeitschr. f. Immunitatsforschung u. exper. Therapie. **65:** 240–244.

Makari, J. G. 1946. Intradermal Test in Malaria. Jour. Trop. Med. and Hyg. **49:** 23–29.

Mayer, M. M., and Heidelberger, M. 1946. Studies in Human Malaria; Complement-Fixation Reactions. Jour. Immunol. **54,** 89–102.

Rich, A. R., and Lewis, M. R. 1932. The Nature of Allergy in Tuberculosis as Revealed by Tissue Culture Studies. Bull. Johns Hopk. Hosp. **50:** 115–131.

Sinton, J. A., and Mulligan, H. W. 1932. Studies in Immunity in Malaria; an Intradermal Reaction in Malarial Infections in Monkeys. Records of the Malaria Survey of India. **3:** 323–345.

Taliaferro, W. H., and Bloom, W. 1945. Inflammatory Reactions in the Skin of Normal and Immune Canaries and Monkeys after the Local Injection of Malarial Blood. Jour. Infect. Dis. **77:** 109–138.

Topley and Wilson's "Principles of Bacteriology and Immunity." 3rd edition, 1946. The Williams & Wilkins Co., Baltimore, vol. 2, chapter 51, p. 1139.

Zuckerman, A. 1945. In Vitro Opsonic Tests with *Plasmodium gallinaceum* and *P. lophurae*. Jour. Infect. Dis. **77:** 28–57.

THE INCIDENCE OF MALARIA AT HIGH ALTITUDES

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The problem of malaria at high altitudes in mountainous country presents a number of curious features, which have attracted attention in recent years. Perhaps the most important point has been the extension of the disease to higher and higher levels, as in the Andes, in East Africa, and in Middle Asia. These regions appear to provide the record altitudes.

Andean malaria has been investigated by Hackett (1945) who demonstrated transmission of the disease by *Anopheles pseudopunctipennis* at 2,600 metres in Bolivia. The prevalence of this mosquito is closely associated with the incidence of malaria throughout the Andes. It behaves differently, however further North and Hackett suggests that the name *pseudopunctipennis* probably includes a complex of closely related forms, some of which are not malaria vectors. This author comes to the conclusion that the maximum elevation at which the disease occurs is no more than a measure of the vertical range of the local vector, and is not usually determined by the limiting effect of cold on sporogony in the mosquito. He also mentions another factor which very likely plays an important part—and that is the sparsity of the human population at the higher levels; this naturally tends to interrupt the cycle.

In southern Tajikistan severe malaria has been reported by Polumordvinov (1945). Epidemics occur in the village settlements between 2200 and 2850 metres, and careful enquiry proved that some of the cases had not left these high regions for 2 years previously. All three forms of malaria were seen and there were many fatalities. The spleen rate in October was as high as 65 per cent in a place of 2750 m. altitude. *Anopheles superpictus* was found at 2400 m. and is probably the vector in this region. The construction of irrigation networks was thought to be responsible for much of the malaria.

The situation in Africa has been analysed by Schwetz (1942) who collected the evidence for autochthonous malaria and related it to the local species of *Anopheles* in the various territories. He gives the altitude limit (for the Congo) as lying between 1700 and 1800 metres, stressing the fact that cases reported above this were probably contracted in the valleys below. He suggests the following theories to explain the absence of malaria above the known limit:

1. The absence also of all species of anopheline mosquitoes; but this is incorrect, some *Anopheles* extend up to 3300 metres.

2. The low temperature prevents transmission from taking place.

3. The anophelines belong to non-vector species; e.g., he states that (in the Congo) above 1700 metres, only *A. kingi*, *A. christyi* and *A. demeilloni* are found.

Schwetz favours the last theory. Other workers in the Congo—Wincke and Jadin (1946)—have shown that the recent extension of malaria into the highest parts of the Ruanda Urundi was due to the introduction of *A. gambiae* following swamp cultiva-

tion. In Kenya, I (Garnham (1945)) reported that annual epidemics were recurring at 2250 m. and that a sharp outbreak took place in 1944 on a farm actually 2550 m. high.

General opinionis summarised by Russell, West and Manwell (1946) in their statement that altitude sharply limits the recurrence of reproduction of various anopheline species, the malaria carriers tending to disappear at the higher places. There are however other factors, and it is the purpose of this paper to describe some of them, particularly in relation to the insidious creep of the disease into hitherto unaffected highlands. Kenya Colony, with its widely varying altitudes, provides a good example of the changing conditions since the development of the country began. Before the first world war, malaria was probably largely absent from all places above 1500 m. and was rare in Nairobi, the capital. Subsequent to 1918, epidemics occured both in Nairobi and in the large farming district around Eldoret (2040 m.); then a few years later other highland districts became infected, culminating in the epidemics during the second world war in the Londiani district (2250–2490 m. Figs. 1 and 2). The number of cases of malaria admitted to hospitals in certain highland districts of Kenya (as shown in Table 1) illustrate how the disease has increased.

What was the cause of this extension of the disease? The answer unquestionably lies in the gradual development of the country. Motor and rail transport brought up the mosquito from the low lying hyperendemic areas. The introduction of the ox wagon meant that rough cart roads had to be made, and in the wheel ruts appeared pools ideal for the breeding of the vector mosquito. The opening of big estates led to much interference with the land, and the production of suitable breeding places. Later, the construction of motor roads and railways was accompanied by the fatal borrow pit. General deforestation increased the facilities for mosquito breeding in sunlit pools. Mill dams on rivers interfered with the natural drainage. Such instances can be multiplied indefinitely, and finally there was the importation of infected labour, which set up foci of carriers everywhere, on the estates, in road gangs, railway construction works, etc. During the second world war, the conditions were intensified by the increased movements and by the erection of military camps. Were anopheline vectors present in these areas before development started? Records are scanty; I think the answer is yes, but that they were in much smaller numbers; in fact so few and far between that there was no transmission of malaria at that time.

Before coming to a description of some typical high altitude epidemics, I should like to emphasise two important points in technique. First, the origin of cases of malaria has to be very carefully investigated. It may frequently happen that the cold of the mountainous district brings out a relapse in a visitor from elsewhere; then the true residents of the high region often descend to lower and more malarious districts, where they contract the disease, which develops on their return home. All these cases must be excluded in any investigation. The second point concerns the transience of conditions. Generally speaking, the higher the locality, the shorter the malaria season. In other words, anopheline surveys must be carried out during a period which may be not more than a few weeks—in all the rest of the year, the results will be barren. Not only is the season short, but the density of the vector

FIG. 1

FIG. 2

FIG. 1, 2. TYPICAL HIGHLAND COUNTRY IN KENYA, SUBJECT TO OCCASIONAL WAVES OF EPIDEMIC MALARIA. ALTITUDE 2400 M.

species is excessively low, even at the peak of transmission. In hyperendemic areas, a single hut catch will often yield a hundred or many more anophelines; here, the

maximum catch that I have obtained is less than 20; it is normally only two or three or frequently none. (These numbers refer to places above 1950 m.)

EPIDEMICS AT HIGH ALTITUDES

Malaria often goes unrecognised in places hitherto free of the disease, and for this reason and because of the absence of early treatment, pernicious forms are particularly liable to be encountered. Reports come in of epidemics of meningitis, of a deadly form of "Spanish flu," or of malignant dysentery. The meningitis is cerebral malaria, the "flu" is severe malignant tertian and the dysentery, the intestinal syndrome of pernicious malaria—all easily diagnosed when the blood slide is examined, but usually too late to prevent a number of fatalities. Even when the reurrence of annual epidemics has familiarised the population with the disease, the community may be so stricken that life comes nearly to a standstill. Practically every bed in the hospitals is occupied by cases of malaria; cultivation of the farm lands ceases and the crops become overgrown with weeds; milking of valuable herds can only be continued

TABLE 1

Hospital admissions for malaria in some districts in Kenya from 1938 to 1946

YEAR	KISII (1680 M.)	KERICHO (1950 M.)	NANDI (1950 M.)
1938	169	520	52
1939	560	357	22
1940	1,895	518	132
1941	780	540	102
1942	1,836	2,428	1,431
1943	1,966	1,703	593
1944	3,486	783	2,710
1945	4,309	1,336	1,184
1946	6,425	2,468	1,684

by importation of labour; in fact, nearly all work stops. Fortunately, the epidemic season is short and the survivors, after about 2 or 3 months' convalescence apparently regain their normal health.

In the highlands of Kenya, the chief causative organism is *Plasmodium falciparum*, and it is this parasite which is responsible for the epidemics. *Plasmodium vivax* is occasionally seen, usually in association with Indian immigrants. *Plasmodium malariae* occurs in a small amount only.

The typical epidemic begins abruptly, usually late in May or early in June, following the "long rains." It reaches its peak in June or July and is on the decline by August. I have never known a true epidemic to occur in any other season in these highlands of Kenya. Fig. 3 shows a severe epidemic which occurred at Kisii (1,680 m.) during 1946. The epidemic curve is similar in places a thousand feet higher, e.g. Kericho. Parasite rates obtained before and after an outbreak give a minimal figure of the incidence. In 1946, at Kericho, three hundred people had a parasite rate of 8 per cent before the epidemic and this figure rose to 36 per cent in 250 examinations at the end of it. Nearly all the infections were due to *P. falciparum*,

only one quartan case was seen and only two benign tertain. Even at the end of the epidemic, the crescent rate was as low as 5 per cent. In age groups, the parasite rates were as follows:—

	Per cent
Under 1 year	10
1–10 years	44
11–15 years	50
Adults	32

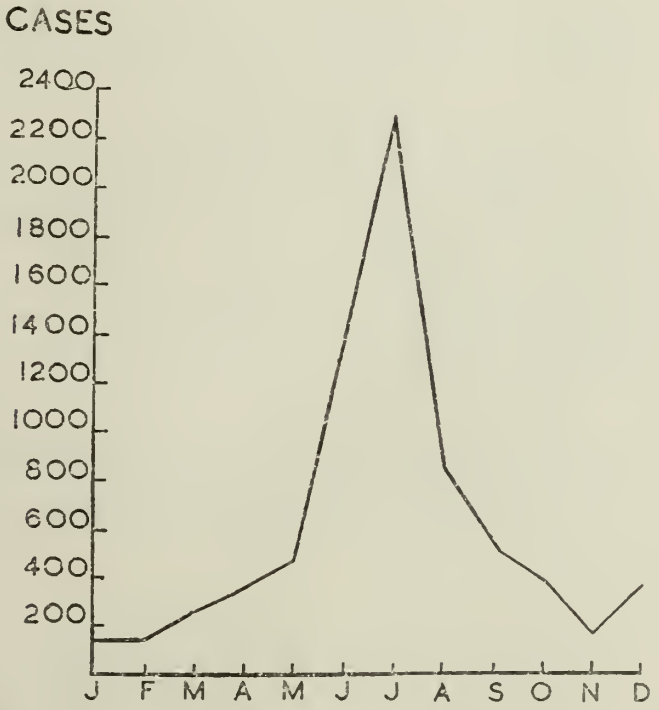

FIG. 3. MALARIA MORBIDITY DURING A SEVERE EPIDEMIC AT KISII, KENYA (1680 M.) DURING 1946

At higher altitudes still, the epidemics are even more restricted in scope, until at the highest their occurrence is more or less an unlucky accident. A good example of the latter was provided by a small farm nestling under the shadow of Timboroa, one of the highest mountains in the Kenya highlands. The farm itself lay between 2,490 and 2,550 m. in altitude, and on it were over 200 Africans. These people suffered from a sharp outbreak of *falciparum* malaria in 1944 and one woman died. The cycle of events was reconstructed as follows: In March, *Anopheles gambiae* mosquitoes were brought to the farm by lorry from an intensely malarious valley 20 miles below. These laid eggs in the numerous suitable breeding places and the first and only generation emerged early in April. The adults bit gametocyte carriers and became infective about the middle of May. Human cases followed eleven days

later and continued to arise for about a fortnight when the last infective mosquito died. Climate records showed that the mean temperature of 61°F.—i.e. the limit for *gambiae* breeding—occurs in this locality only in one month of the year, March, so it is evident that circumstances such as these are unlikely to arise very often.

The epidemics I have just described, affected populations highly susceptible to malaria, and were very largely due to *A. gambiae*. In other conditions the picture may be very different. Early in 1947 Dr. and Mrs. Bagster Wilson and I, investigated a very interesting outbreak of the disease on Lake Bunyonyi in Uganda (Garnham, Wilson & Wilson, 1948). This lake lies at a height of 1,920 m. on the mountainous frontier between Uganda and Ruanda-Urundi. The anopheline vector appears to be solely *A. funestus*; *gambiae* is apparently absent. It breeds in the papyrus choked bays of the lake, and the adults are found in large numbers in the neighbouring huts. As many as 170 females were collected on one occasion from 3 huts. Malaria is rampant amongst the inhabitants; it shows little seasonal change. The parasite rate is extremly high—86 per cent in children between 1 and 5 years, whilst the spleen rate in the adults is most exceptional—75 per cent, although their parasite rate is only 25 per cent. The dominant species was *P. falciparum*, but 10 per cent of the population was also infected with *P. malariae*.

The occurrence of malaria at high altitudes must be greatly influenced by the mean temperature of the locality. With every 100 metres, the temperature is stated by Loewy and Wittkower (1937) to fall by half a degree C, irrespective of the latitude or the continent. Other factors play a minor part, for instance these same authors point out that in high valleys exposed to wind, mosquitoes are unable to penetrate high, whilst on the opposite side of the range, where calm weather prevails they will ascend to a much greater altitude. Then the steeper slopes higher up are less likely to provide suitable breeding places for the anopheline vector.

The temperature acts in two ways:

1. It affects the ability of the mosquito to complete its cycle.
2. It affects the ability of the parasite to develop in the mosquito.

I propose to discuss these two points in a little detail as they represent the crux of the problem of highland malaria. The observations refer to East African conditions

THE MOSQUITO CYCLE

It is well known that mountainous districts in the tropics have a mosquito fauna peculiar to the altitude. Edwards (1941) refers to the distinctive species which inhabit the "humid montane province" and mentions *Anopheles ardensis*, *natalensis*, *garnhami*, *cinereus*, *kingi*, *christyi* and others. None of these, with the possible exception of the last one, are malaria carriers in nature and it is the intrusion of that pair of dangerous vectors—*A. gambiae* and *A. funestus*—into the highlands, that has created the problem. The mosquitoes in the former list find no difficulty in completing their cycle in the cold regions, but it is quite another matter with the last two. The mean atmospheric temperature below which these species will not breed is stated by de Meillon (1934) to be 61°F., though the minimum temperature of the breeding waters is much lower—about 53°F.

Some typical temperatures in places in Kenya are shown in Table 2; at 2,700 m.

the temperature never reaches 61°F. and malaria is absent; at 2,250 m. this temperature occurs only during four months of the year and it is following these months that malaria is epidemic. Slightly higher, where a temperature of 61°F. is only attained for a single month in the year, exceptional epidemics may occur if a vector species happens to be introduced. At Londiani (2,250 m.) larvae of *A. gambiae* can usually only be recovered in April, May and June and adults of the species in June and July; a thousand feet lower, the larval season extends from March to July, the adults prevailing in May, June and July. The actual hut density is remarkably low, as Table 3 shows.

The rise in altitude and the drop in temperature appears to affect anopheline breeding principally by lengthening the duration of the cycle. This was tested by collect-

TABLE 2

Mean monthly temperatures, Kenya Colony

PLACE	ALTITUDE IN METRES	TYPE OF MALARIA	JAN.	FEB.	MAR.	APR.	MAY.	JUN.	JUL.	AUG.	SEP.	OCT.	NOV.	DEC.
			°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Kisumu	1,140	Hyperendemic	77	76	75	73	73	72	72	72	73	75	75	76
Nairobi	1,650	Endemic	64	65	65	64	63	62	59	59	62	65	64	62
Londiani	2,250	Epidemic	59	61	63	62	61	59	57	56	58	59	59	59
Equator	2,700	Absent	57	58	59	57	57	56	53	54	55	55	55	55

TABLE 3

Number of A. gambiae *females found in huts (altitude 1,950 m.) from April to October*

MONTH	NO. OF HUTS EXAMINED	ANOPHELES PER HUT PER SEARCH
April	104	Nil
May	218	0.3
June	42	2
July	178	4
August	7	2
September	45	Nil
October	120	Nil

ing the eggs from local *A. gambiae* females and allowing them to hatch in dishes. The young larvae were transferred to basins sunk in the ground and exposed to sun light. The average length of the cycle at 1,950 m. was as follows:

	days
May	20
June	24
July	24
August	24
September	27

During a hot dry spell, one batch completed the cycle in 17 days. At Kisumu, nearly a thousand metres lower, the average cycle is 11 days; in Nairobi (1,650 m.)

Symes (1941) found it to be 18 days. At Londiani which at 2,250 m. represents nearly the limit of breeding, the cycle is probably a month.

Another factor which influences anopheline breeding is the amount of rainfall. The temperature may be high enough for successful breeding (e.g. Londiani in February—63°F.) but low precipitation will prevent it. The actual amount necessary depends on the nature of the locality, but it is probably about 6 inches in the month. It is not proposed to discuss this further, as it is not a factor peculiar to high altitude malaria.

THE DEVELOPMENT OF THE PARASITE IN THE MOSQUITO

Wenyon (1926) states that a minimum constant temperature of 64.4°F. is necessary for the development of *P. falciparum*. In most of these high epidemic localities,

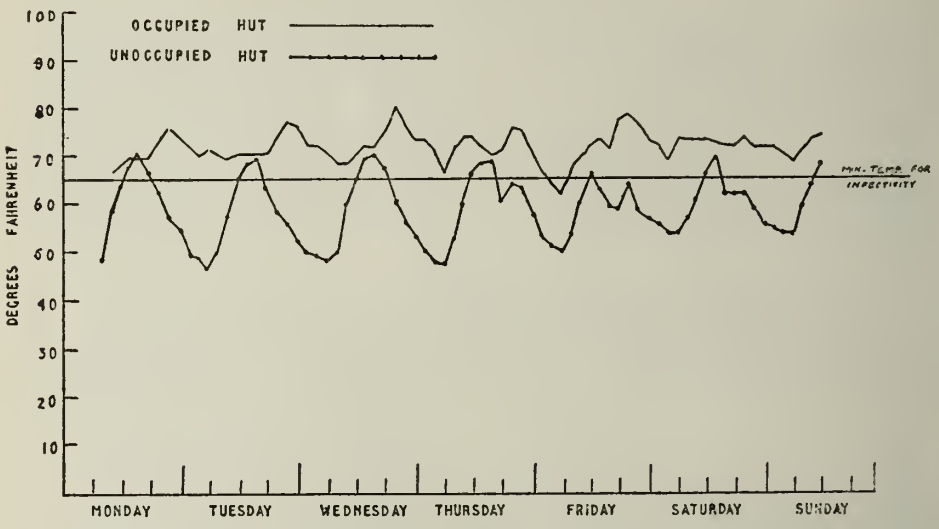

Fig. 4. Thermograph Tracings Inside Occupied and Unoccupied Huts
Note how the minimum temperature for infectivity (64.4°F.) divides the two tracings.

such a temperature is never reached. The explanation of the discrepancy is to be found in the habits of the adult vector mosquitoes. They spend the major part of their life indoors—preferably in the small thatched native hut, with no windows or ventilation, a small low door, a constant fire and overcrowded with people and domestic animals. Observations on comparative temperatures at 2,250 m. indicated that the hut is usually at least 5°F. warmer than outside. At 1,950 m. some thermograph tracings were obtained from occupied and unoccupied huts and Fig. 4 gives a typical comparison.

The horizontal line at 64.4°F. represents the minimum constant temperature necessary for the development of *P. falciparum* and it almost exactly divides the two tracings. In other words, an occupied hut is suitable for sporogony, whilst an unoccupied one is not. Outside harbourages of anopheline mosquitoes are comparable with the latter and these findings, which have been confirmed in two successive years,

suggest that if a mosquito is to become infective, it must spend practically all its time inside an occupied hut. It probably makes flights to a pool of water for oviposition, but rapidly returns to the warm shelter of a hut.

Saturation deficiency outside huts is also unfavourable to the survival of the mosquito. Observations taken in a Stevenson screen frequently showed a daily range between 12 per cent and 80 per cent relative humidity. In an unoccupied hut, there was a daily swing between 40 per cent and 85 per cent. The humidity in an occupied hut on the other hand was much more steady, fluctuating around 60 per cent—obviously providing a more suitable environment for the insect.

Dissection of mosquitoes caught in these localities demonstrated infection of the salivary glands. Four out of 287 *A. gambiae* showed sporozoites, (3 positives from a place 1,950 m., the fourth from 2,250 m.) in 1946. In 1947, Dr. R. B. Heisch gives me (in litt.) the following records:

1,251 *A. gambiae* dissected—16 positive i.e. 1.3 per cent.

232 *A. funestus* dissected—4 positive i.e. 1.7 per cent.

CONCLUSIONS

The special points I want to stress in this paper are two; first, the ravages which malaria is causing by creeping higher and higher into the so-called healthy highlands of East Africa. This is apparently the result of the development of the country, which leads to the creation of numerous breeding places of the carrier anophelines, provides transport to bring the mosquitoes up, and introduces a large infected population to carry out the various works.

The second point of importance is that connected with the meteorological findings. It is apparently only because *A. gambiae* and *A. funestus* are essentially hut insects that malaria occurs at all at places over 1,500 m. or so. It is even possible that in the higher places, malaria could be eliminated by abolishing fires from all the huts and installing outside kitchens. Then the mosquito could never find an environment warm enough for it to develop a gland infection. A further implication is that the use of an insecticide such as D.D.T. should be effective in these circumstances. If it failed to kill all the resting adults (as suggested by Muirhead Thomson, 1947) it would at least drive them into the inclement exterior where sporogony would forthwith cease.

ACKNOWLEDGMENT

I have the pleasure to acknowledge with gratitude the help afforded me by Mr. J. O. Harper throughout the many years in which the mosquito observations were made.

REFERENCES

De Meillon, B. 1934 Observations on *Anopheles funestus* and *Anopheles gambiae* in the Transvaal. Publ. S. A. Inst. Med. Res. **6**: pp. 195–248.

Edwards, F. W. 1941 Mosquitoes of the Ethiopian Region. III. Brit. Mus. London.

Garnham, P. C. C. 1945 Malaria Epidemics at exceptionally high altitudes in Kenya. Brit. Med. Jl. **11**: pp. 45 and 46.

Garnham, P. C. C., Wilson, D. B., and Wilson, M. E. 1948 Malaria in Kigezi, Uganda. J. Trop. Med. & Hyg. **51**: pp. 156–159.

Hackett, L. W. 1945. The Malaria of the Andean region of South America. Rev. Inst. Salubr. Enferm. Trop. **6:** pp. 239–252.

Loewy, A. and Wittkower, E. 1937 The Pathology of High Altitude Climate. Oxford University Press, London.

Polumordvinov, A. D. 1945 Highland Malaria in Southern Tadjikistan. Med. Parasit. Parasit. Dis. Moscow. **14:** pp. 18–20.

Russell, P. F., West, L. G. and Manwell, R. D. 1946 Practical Malariology. W. B. Saunders & Co., Philadelphia and London.

Schwetz, J. 1942 Recherches sur la Limite Altimetrique du Paludisme Dans le Congo Orientale et sur la Cause de Cette Limite. Ann. Soc. Belge. Med. Trop. **22:** pp. 183–209.

Symes, C. B. 1941 Malaria in Nairobi. E. Afr. Med. J. **17:** pp. 414–430.

Thomson, R. C. M. 1947 Recent knowledge about malaria vectors in West Africa and Their Control. Trans. Roy. Soc. Trop. Med. & Hyg. **40:** pp. 525–536

Wenyon, C. M. 1926 Protozoology. Vol. II: Baillere, Tindal & Cox, London.

Wincke, I. H. and Jadin, J. B. 1946 Contribution a l'etude de l'anophelisme en pays d'altitude. Ann. Soc. Belge. Med. Trop. **26:** pp. 483–500.

AMERICAN FOUNDATION OF TROPICAL MEDICINE MAKES GRANT TO JOURNAL

The Editorial Committee of the Journal acknowledges the generous grant from the American Foundation of Tropical Medicine of $1000 for their publication fund. In common with other publications the Journal has been limited in size because of the higher costs of publishing. This grant will permit extension of the size of the Journal to include papers that would otherwise have been rejected on account of lack of space.

MOSQUITO CONTROL ASSOCIATIONS HOLD JOINT CONFERENCE—FEB. 6 TO 9

The California Mosquito Control Association will hold its annual conference jointly with the American Mosquito Control Association at Berkeley and Oakland, California, February 6 to 9, 1949. The program will consist of one day each being devoted to speakers and topics of international, national and local California problems affecting mosquito control agencies. The meeting will be followed by a field trip (February 10–15) to observe the work and problems of a number of California Mosquito Abatement Districts as well as to visit some of the attractions of California. Invitation to attend is open to all interested.

MALARIA MORTALITY AND MORBIDITY IN THE UNITED STATES FOR THE YEAR 1946*

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This report is a continuation of the yearly statistical analysis of malaria in the United States, first undertaken by the senior writer beginning in 1930 (Faust, 1932). For the first few years of the reports only mortality data for the highly malarious southeastern states were considered. Then contiguous political units were added and beginning in 1940 a yearly assay was undertaken of malaria deaths and morbidity for the entire United States.

Although malaria in the United States was appreciably less in 1930 than it had been for nearly a century (and was manifesting evidence of continued reduction), the period from 1931 through 1938 provided a considerable upsurge of the disease. This corresponded to the cyclic rise which had been noted approximately every five to seven years, but was more intense and extended more into the periphery of the highly endemic southeastern area than had been anticipated. Competent malariologists attributed this increase primarily to the economic depression with which the greater malariousness corresponded. Thereafter, as a result of intensive antimalarial activity, the amount of the disease rapidly declined, no additional cyclic recrudescences occurred, and as of the end of 1946 autochthonous malaria had decreased to a previously unreported low level.

Meanwhile many hundreds of thousands of persons in the armed services of the United States had contracted malaria in several theaters of military operations overseas and an appreciable number of these individuals had returned home with relapsing malaria, for the most part vivax infection. This produced a potential hazard for population groups in many areas to which the infected individuals returned, since it had been demonstrated that the important anopheline transmitters of autochthonous strains of vivax, quartan and falciparum malaria parasites in the United States were equally capable of transmitting exotic strains. Moreover, the presence of cases of malaria acquired outside the Continental United States complicated the statistical picture, since reports were now coming in with appreciable numbers of cases appearing in areas which had been malaria-free for many years or at most had only few sporadic cases. In the previous report by this Committee (Faust, Scott and McDaniel, 1947) an attempt was made to separate native from introduced malaria but the data obtained from state bureaus of vital statisics were not satisfactory for analysis.

* Report of the Committee on Statistics, National Malaria Society, Atlanta, Georgia, December 3, 1947.

In 1946 the majority of states for the first time reported cases of malaria on the basis of the source of infection, viz. "inside" or "outside" the Continental United States. Even though much of the morbidity data is not satisfactory due to such categories as "sources unknown" or "sources not designated," it will be seen subsequently that the division of cases is on the whole helpful in obtaining an overall picture of malaria for the year.

The mortality data have been made available to this Committee by the U. S. Department of Commerce, Bureau of the Census and by the Federal Security Agency, U. S. Public Health Service, Office of Vital Statistics. The basic morbidity data have been obtained through the courtesy of the Federal Security Agency, U. S. Public Health Service, Division of Public Health Methods, and have been supplemented by returns from special inquiries to state health departments. Grateful acknowledgment is made to all services and individuals providing the records on which this report is based, and particularly to the Statistical Branch of the Communicable Disease Center of the U. S. Public Health Service for tabulating the records (Tables 1, 2) and drafting the maps (Figs. 1–3).

PRESENTATION OF DATA

Mortality Data

The reported malaria deaths year by year by states, 1932–1946, in association with malaria morbidity for the same categories, are summarized in Table I.

A total of 341 deaths from malaria were reported for residents in the Continental United States for the year 1946. This constitutes a reduction of 23 per cent from 1945 (443), 41.2 per cent from 1944 (580), 47.3 per cent from 1943 (647) and 92.5 per cent from 1933 (4678), the peak year of the last cyclic increase in malaria. With the possible exception of Texas (63 deaths for 1946 vs. 97 for 1942, 68 for 1943, 82 for 1944 and 52 for 1945), the decline in the number of deaths in each of the malarious states is noteworthy and is in line with the general decline and total trend.

Fig. 1 provides a spot distribution by counties of the number of reported malaria deaths for 1946. These include both autochthonous and exotically acquired infections. Deaths reported from New Hampshire, New York, Pennsylvania, Michigan, Minnesota, Iowa, Nebraska, Kansas, Wyoming, Montana, Nevada and California most probably resulted from infection acquired overseas or in the more heavily endemic areas in the southeastern states. Because of the extensive scatter of the localities in which deaths occurred it would not be useful to compute the rates.

Deaths were reported from 235 counties. In 178 counties only one death each occurred; in 36 counties, 2 deaths each; in 12 counties, 3 deaths each, and in 9 counties (Orangeburg Co., S. C., Shelby Co., Tenn., Bolivar Co., Miss., Jefferson and Phillips Cos., Ark., Tulsa Co., Okla., Montgomery and Hidalgo Cos., Tex., and Los Angeles Co., Calif.) 4 or more deaths each. As in previous years a high percentage of the deaths and of the counties in which they occurred was in the southeastern area and the adjacent eastern third of Texas and Oklahoma. This is particularly evident with respect to the location of counties with more than one death.

TABLE 1

*United States malaria mortality and morbidity**

By state, by year

STATE	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946
Alabama															
Deaths	179	265	292	322	350	219	225	198	204	123	90	56	39	37	30
Cases	2203	4509	6473	8632	8438	4599	6006	6986	9442	4835	4367	3230	2882	2913	1541
Arizona															
Deaths	1		2		1	3		1						1	2
Cases	5	14	15	36	57	40	29	27	35	46	35	82	61	189	236
Arkansas															
Deaths	456	869	599	571	407	384	371	265	176	164	118	107	87	43	25
Cases	1513	3745	1949	3587	1748	4462	5480	4863	3511	3426	1975	1156	1452	2262	1340
California															
Deaths	9	3	6	5	6	10	11		5	2	2	6	9	3	7
Cases	45	63	182	171	184	170	337	286	168	160	83	2050	1693	1915	1222
Colorado															
Deaths	1	2		1		4	1			1	2	1	1	1	
Cases	5		2	2		1	5	4	3	2	3	30	36	846	86
Connecticut															
Deaths		1		1			1							1	
Cases	3	4	4	5	7	5		6	5	10	M	20	60	300	456
Delaware															
Deaths															
Cases	M	M	3		10	1	6			1			15	48	12
District of Columbia															
Deaths		1	1	1				1	1	1				1	1
Cases											5	45	171	144	27
Florida															
Deaths	246	382	453	326	345	204	162	109	98	82	50	44	33	21	20
Cases	318	993	1109	813	869	953	472	447	148	141	86	111	496	797	495
Georgia															
Deaths	315	367	410	381	612	242	205	118	107	83	79	43	39	24	14
Cases	2969	4264	3904	3749	9705	5761	3463	2731	2258	1065	905	491	373	915	583
Idaho															
Deaths				2				1	1						
Cases						4		1	6		4	2	4	39	66
Illinois															
Deaths	37	61	57	66	53	38	35	36	22	7	14	11	6	9	14
Cases	129	250	322	378	223	207	264	481	199	88	89	181	23	13	565
Indiana															
Deaths	10	18	17	10	5	21	8	6	8	7	6	4	3	4	3
Cases	1	17	7	27	5	6	71	60	28	18	M	281	430	645	344
Iowa															
Deaths	1	4	1	1	3	2	2	1	1			1	2	3	4
Cases	M	2	4	22	12	12	13	62	60	21	M	16	241	465	280
Kansas															
Deaths	8	8	6	5	6	3	3	1	5	2		4	1	3	2
Cases	6	16	21	51	20	24	20	32	23	18	11	45	160	781	61
Kentucky															
Deaths	35	79	63	71	51	38	30	29	19	14	13	11	16	5	6
Cases	133	294	204	296	110	91	100	103	48	25	36	69	80	1071	329
Louisiana															
Deaths	156	428	363	361	249	180	184	111	87	77	57	37	28	33	22
Cases	881	2915	2737	3649	1614	1115	698	584	519	391	360	302	1486	1327	745
Maine															
Deaths				1		1			1		1		1	1	
Cases	1				3	4		1	1	1	M	4	4	31	88
Maryland															
Deaths	1	2	1	3	1		2				1	1		1	1
Cases	8	15	11	44	27	16	22	5	14	22	13	12	11	586	73
Massachusetts															
Deaths	2		2	1		2	1	2		1	3	2	6	3	
Cases	14	28	27	17	13	14	13	14	7	11	18	118	625	1028	497

TABLE 1—*Continued*

STATE	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946
Michigan															
Deaths	1	2	5	9	4	4	1	4	1	6	4	4	1	10	3
Cases	44	63	104	82	82	115	58	66	60	24	24	257	244	469	1306
Minnesota															
Deaths		1		2	1					1	4	2	2	1	1
Cases		2	2	3	8	3	4	22	10	4	1	2	46	296	999
Mississippi															
Deaths	395	807	688	531	362	312	275	235	174	150	79	67	63	37	29
Cases	36528	72497	75555	70350	57609	47826	44282	44237	40962	36039	30699	24284	22838	18764	17387
Missouri															
Deaths	116	197	186	164	104	89	75	66	41	25	22	19	15	13	6
Cases	138	241	1488	2000	1144	1277	451	137	100	59	77	115	223	426	354
Montana															
Deaths			1		1				1						1
Cases			1	1	3			3	1	1	M	6	28	32	10
Nebraska															
Deaths	1	2	1	3		1			1				1	1	2
Cases	1									1	1	4	2	13	157
Nevada															
Deaths	1	1										1			1
Cases			1					M	1		M	4	4	5	14
New Hampshire															
Deaths	3		1	1	1							1			2
Cases										2			2	4	17
New Jersey															
Deaths	44	1		7	2		3	1	1		3	2	1	4	2
Cases	6	7	19	128	36	26	14	12	14	13	19	16	831	1412	931
New Mexico															
Deaths	6	1	1	4	2		2	6	1			1	3	1	1
Cases	65	147	220	72	73	38	21	23	90	39	14	11	13	70	84
New York															
Deaths	2	18	21	18	9	29	26	20	26	3	7	4	5	7	3
Cases	46	76	102	77	96	113	124	124	134	80	107	104	433	1234	2063
North Carolina															
Deaths	53	51	66	91	150	87	70	53	60	28	33	22	25	24	8
Cases						876	636	632	653	237	209	185	154	554	369
North Dakota															
Deaths			1	1								1			
Cases		M						2			M	3	5	1	5
Ohio															
Deaths	7	10	4	10	9	7	5	4	3	6	4	4	5	5	3
Cases	17	65	104	109	55	25	37	25	49	19	8	39	158	110	414
Oklahoma															
Deaths	109	134	139	139	94	92	90	46	31	54	25	31	19	19	12
Cases	1162	1581	889	2147	1207	1387	1497	1957	1871	1911	1498	1315	1460	1142	500
Oregon															
Deaths	1				1	2		1	1	1	1		2		
Cases	53	24	23	42	63	36	13	14	32	40	46	23	293	54	78
Pennsylvania															
Deaths	10	4	4	5	8	11	5	4	1	3	5	2	1	8	11
Cases	9	12	28	17	23	14	19	13	16	10	10	4	1		1
Rhode Island															
Deaths						1	2	1				1		1	
Cases	1	4							1	2	2	6	215	168	203
South Carolina															
Deaths	234	244	354	430	435	264	216	168	117	125	104	64	64	41	19
Cases	11954	11053	9369	10399	9447	10652	11461	12024	9435	9830	10017	9866	9899	9852	5933
South Dakota															
Deaths														1	1
Cases		3		3		1	1	2				4		7	18
Tennessee															
Deaths	132	275	265	221	156	105	106	96	64	50	31	21	13	20	17
Cases	1804	4392	3466	2807	1828	1183	1403	1471	903	476	313	191	190	190	388

TABLE 1—*Continued*

STATE	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946
Texas															
Deaths	M	429	497	644	495	361	256	170	177	157	97	68	82	52	63
Cases	7107	17238	21758	26343	25373	20025	4400	4540	6606	8067	7678	8266	7498	8969	6799
Utah															
Deaths	1			1		1							1		
Cases	M	M	M	M	M	M	M	1	5		M	313	151	112	93
Vermont															
Deaths							2							1	
Cases	M	M	M	M							M	1		4	
Virginia															
Deaths	6	10	10	16	17	10	2	5	5	3	6	1	1	2	3
Cases	336	262	257	371	498	207	156	149	110	75	54	173	717	832	498
Washington															
Deaths	1		2			1							2		
Cases			3	1	1		5	1	2	8	3	2	1	6	30
West Virginia															
Deaths		1	1	4	1	1	1					1	2	1	
Cases	1	1	10	2	3	6		3	9	7	8		22	175	88
Wisconsin															
Deaths				1	2			2	2	1		2	1		1
Cases							1	3	3	1	M	31	90	188	69
Wyoming															
Deaths				1						1					1
Cases	2				2	1			7	2	3	2	11	7	49
U. S. Totals															
Deaths	2540	4678	4520	4435	3943	2729	2378	1761	1442	1178	861	647	580	443	341
Cases	67508	124797	130373	136433	120596	101296	81582	82154	77549	67228	58781	53482	55832	61411	47903

M Missing data.

* Morbidity:
Federal Security Agency, U. S. Public Health Service,
Office of the Surgeon General, Division of Public Health Methods, and State Health Departments.
Mortality:
Department of Commerce, Bureau of the Census and Federal Security Agency,
U. S. Public Health Service, Office of Vital Statistics.

Morbidity Data

Malaria was reported in 1946 from 47 states and the District of Columbia. Vermont had no record of malaria. The data by states, broken down into four categories, viz., (1) contracted within Continental United States, (2) contracted outside Continental United States, (3) source of exposure unknown and (4) source not designated, are itemized in Table II.

The total of reported cases, after checking with certain northern state bureaus and the health department of Chicago, Illinois, amounts to 47,850. Of this number 34,888, or 72.9 per cent, were stated to be autochthonous and 11,588 or 24.2 per cent to be acquired outside the United States.

For 1946 malaria was reported from 1544 counties, or approximately half of those in the United States. A total of 26.3 per cent of the counties reported one or more cases contracted within the United States and 25.7 per cent reported one or more cases acquired overseas.

Although Vermont alone reported no malaria, there was no locally contracted infection reported for New England except in one county of Connecticut, none in New York except New York City, none in Delaware, West Virginia, Wisconsin, the

TABLE II

Malaria morbidity and rates per 100,000 population with source of disease designation—1946

STATES	CONTRACTED WITHIN U. S.			CONTRACTED OUTSIDE U. S.			SOURCE UNKNOWN			SOURCE NOT DESIGNATED			TOTAL		
	Cases	% in state	Rate	Cases	% in state	Rate	Cases	% in state	Rate	Cases	% in state	Rate	Cases	% in state	Rate
Ala.	1263	82.0	42.8	278	18.0	9.4							1541	100.0	52.2
Ariz.	30	12.7	5.6	198	83.9	36.7	8	3.4	1.5				236	100.0	43.8
Ark.	1197	89.3	59.6	108	8.1	5.4	35	2.6	1.7				1340	100.0	66.7
Cal.	522	42.7	6.8	513	42.0	6.7	187	15.3	2.4				1222	100.0	15.9
Colo.				86	100.0	7.3							86	100.0	7.3
Conn.	1	0.2	0.1	455	99.8	25.7							456	100.0	25.7
Del.				12	100.0	4.2							12	100.0	4.2
D. C.	10	37.0	1.3	17	63.0	2.2							27	100.0	3.5
Fla.	99	20.0	4.6	71	14.3	3.3	325	65.7	15.0				495	100.0	22.9
Ga.	108	18.5	3.3	475	81.5	14.6							583	100.0	17.9
Idaho	1	1.5	0.2	52	78.8	9.0	13	19.7	2.3				66	100.0	11.5
Ill.	14	2.7	0.2	493	96.3	6.1	4	0.8	0.0				512†	100.0	6.3
Ind.	22	6.4	0.6	294	85.5	8.3	28	8.1	0.8				344	100.0	9.7
Iowa	1	0.4	0.0	275	98.2	10.7	4	1.4	0.2				280	100.0	10.9
Kans.				58	95.1	3.3	3	4.9	0.2				61	100.0	3.5
Ky.	44	13.4	1.5	285	86.6	9.5							329	100.0	11.0
La.	567	76.1	22.4	23	3.1	0.9	155	20.8	6.1				745	100.0	29.5
Me.				82	93.2	9.3	6	6.8	0.7				88	100.0	10.0
Md.	2	2.7	0.1	71	97.3	3.7							73	100.0	3.8
Mass.				341	68.6	7.8	104	20.9	2.4	52	10.5	1.2	497	100.0	11.4
Mich.	1	0.1	0.0	1303	99.8	23.6	1	0.1	0.0				1306*	100.0	23.7
Minn.	4	0.4	0.1	995	99.6	33.9							999‡	100.0	34.0
Miss.	17364	99.9	757.4	23	0.1	1.0							17387	100.0	758.4
Mo.	121	34.1	3.1	228	64.4	5.9	5	1.4	0.1				354	100.0	9.1
Mont.				10	100.0	1.7							10	100.0	1.7
Neb.				150	95.5	11.8	7	4.5	0.8				157	100.0	12.3
Nev.				14	100.0	11.5							14	100.0	11.5
N. H.				17	100.0	3.4							17	100.0	3.4
N. J.	7	0.8	0.2	923	99.1	21.8	1	0.1	0.0				931	100.0	22.0
N. M.	14	16.7	2.3	70	83.3	11.7							84	100.0	14.0
N. Y.	9	0.4	0.1	1692	82.0	12.1	362	17.5	2.6				2063	100.0	14.7
N. C.	276	74.8	7.2	93	25.2	2.4							369	100.0	9.7
N. D.				5	100.0	0.8							5	100.0	0.8
Ohio	109	26.3	1.5	305	73.7	4.3							414	100.0	5.9
Okla.	298	59.6	13.0	183	36.6	8.0	19	3.8	0.8				500	100.0	21.8
Ore.				72	92.3	6.1	6	7.7	0.5				78	100.0	6.6
Pa.	1	100.0	0.0										1	100.0	0.0
R. I.				202	99.5	27.7							203*	100.0	27.8
S. C.	5931	100.0	296.5	2	0.0	0.1							5933	100.0	296.6
S. D.				10	55.5	1.6				8	44.4	1.3	18	100.0	2.9
Tenn.	100	25.8	3.2	288	74.2	9.3							388	100.0	12.5
Tex.	6366	93.6	93.8	433	6.4	6.4							6799	100.0	100.2
Utah				93	100.0	16.1							93	100.0	16.1
Vt.													00	—	—
Va.	404	81.1	14.2	73	14.7	2.6	21	4.2	0.7				498	100.0	17.6
Wash.	2	6.7	0.1	22	73.3	1.2	6	20.0	0.3				30	100.0	1.6
W. Va.				87	98.9	4.3	1	1.1	0.0				88	100.0	4.4
Wis.				63	91.3	1.9	6	8.7	0.2				69	100.0	2.1
Wyo.				45	91.8	16.9	4	8.2	1.5				49	100.0	18.4
U. S. Total	34888	72.9	25.4	11588	24.2	8.4	1311	2.7	1.0	60	0.1	0.0	47850	100.0	34.9

* Includes 1 due to blood transfusion.
† Differs from U.S.P.H.S. data by 53 due to change in Cook County, Illinois.
‡ Includes 2 due to blood transfusions.
Rates based on estimated population for 1946.
Revised per E.C.F. 12/24/47 and 3/4/48.

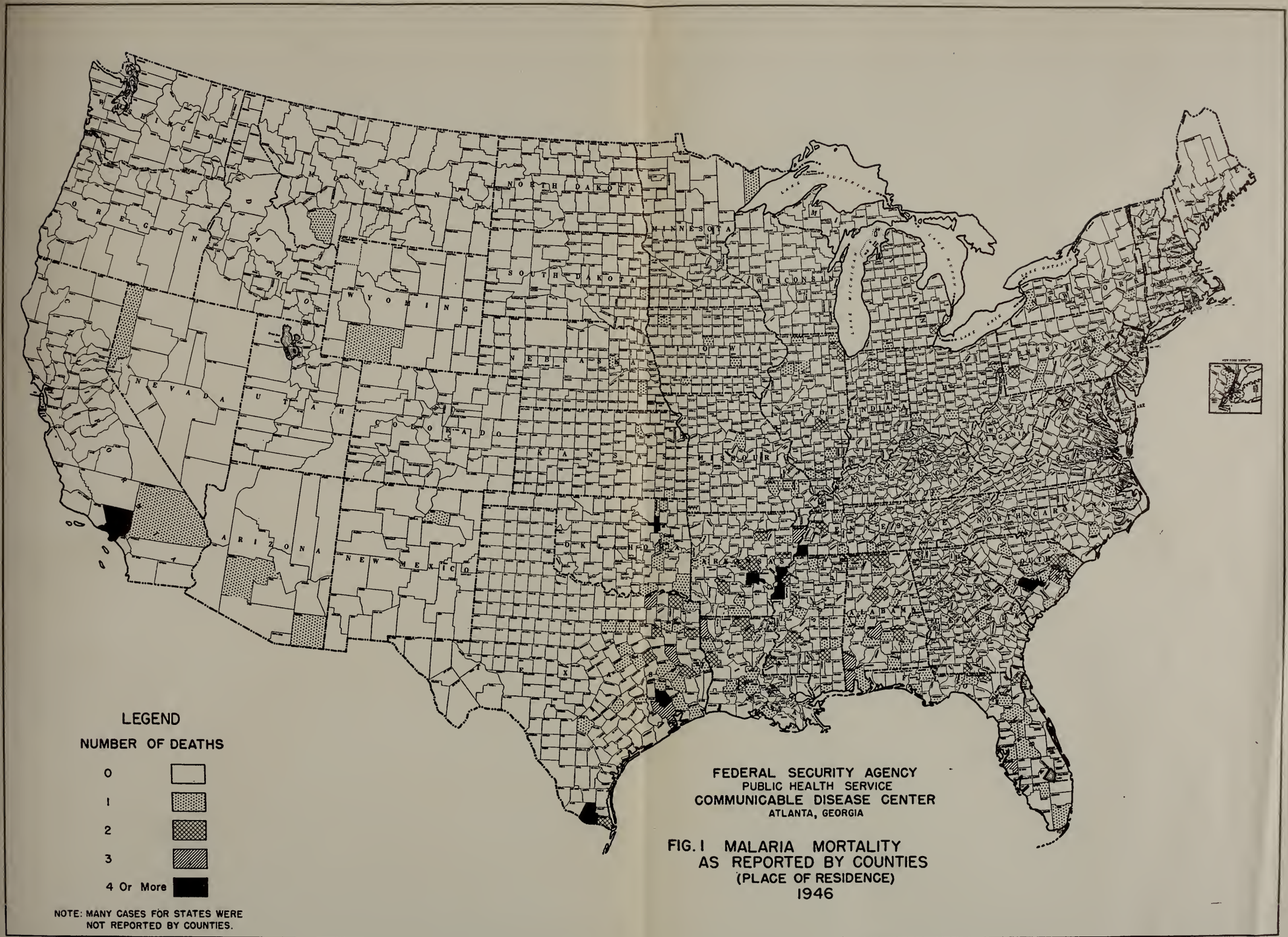

FIG. 1. Map of United States showing number of malaria deaths by counties for 1946.

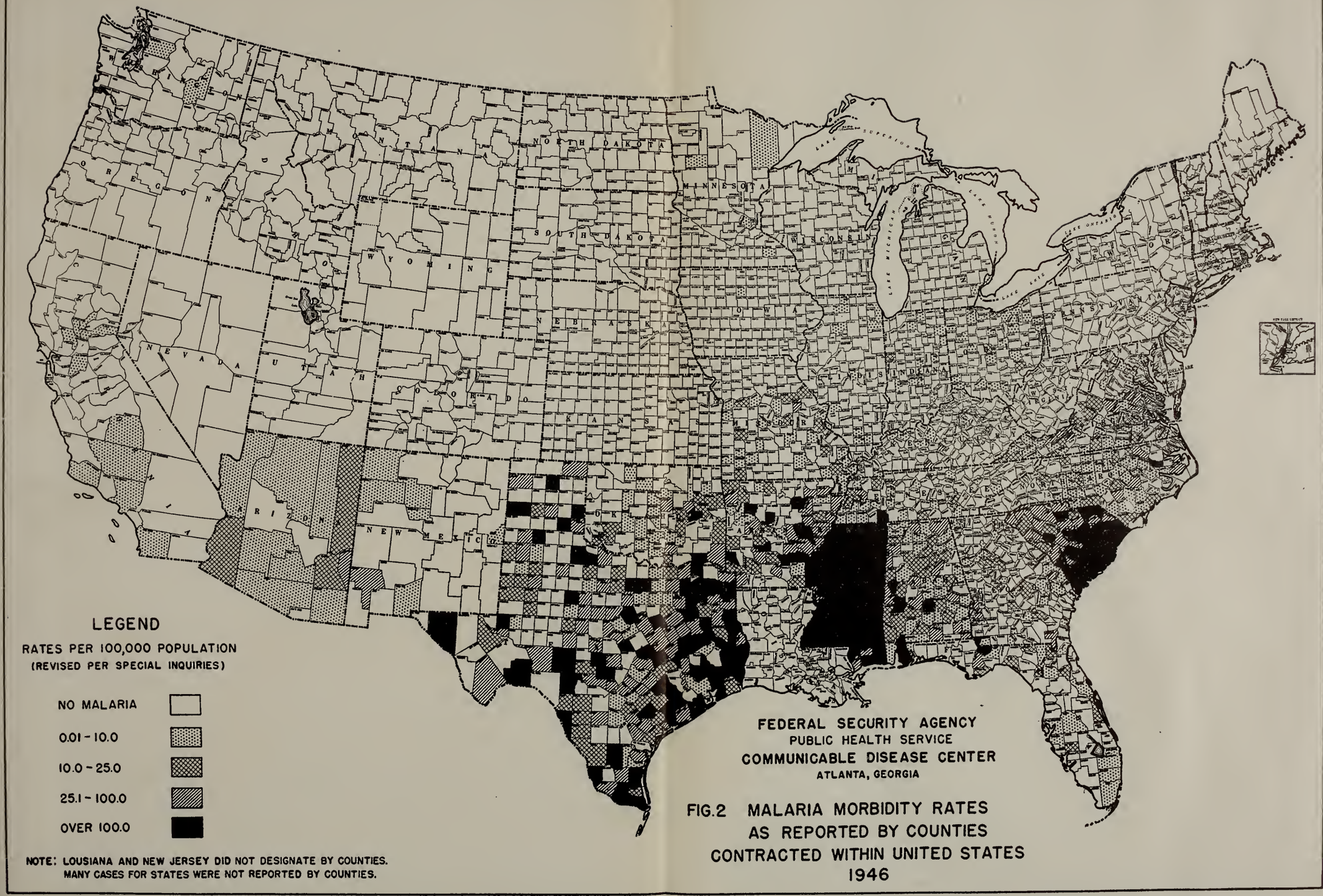

FIG. 2. Map of the United States showing morbidity rates as reported by counties for malaria contracted within the Continental United States.

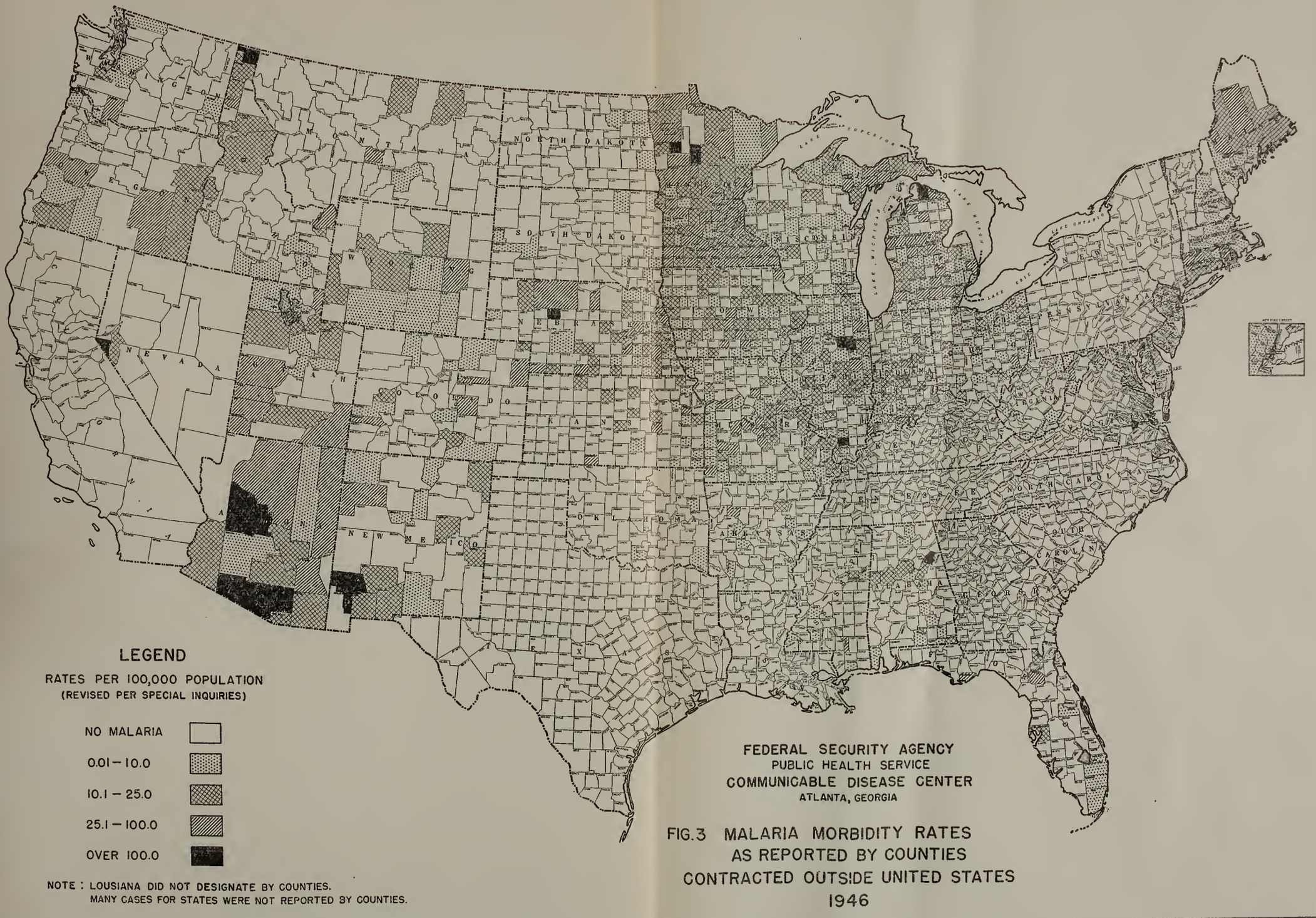

FIG. 3. Map of the United States showing morbidity rates as reported by counties for malaria contracted outside the Continental United States.

Dakotas, Nebraska, Kansas, Montana, Wyoming, Colorado, Idaho, Utah, Nevada or Oregon, and only one county each involved in New Jersey, Maryland, Pennsylvania, Michigan and Iowa. While a few cases of locally contracted infection are distributed throughout the northern states from Minnesota to the east coast, the areas of contiguous counties where malaria reportedly prevails consist of Virginia and the Carolinas, and a block of states from eastern Alabama through to western Texas.

The county rates per 100,000 population for malaria reportedly contracted within the United States are shown on the accompanying map (Fig. 2). Predominant foci appear in South Carolina, Mississippi, eastern Texas and to a somewhat lesser extent in Virginia, eastern North Carolina, Alabama, Arkansas, eastern Oklahoma, central and western Texas and Arizona. If Louisiana had been able to provide data by parishes, the northern part of this state would also have been included in this group of moderately endemic foci.

Malaria acquired outside the confines of the United States and reported in 1946 shows a predominant frequency in New England, New York, New Jersey, the North-Central States, the Mountain States, Oregon and Washington, for the most part almost completely outside the present endemic areas. In contrast, such southern states as Virginia, North Carolina, Alabama, Arkansas, Louisiana, Oklahoma and Texas reported only a minor fraction of the total malaria morbidity as contracted outside the United States, while South Carolina recorded 99.9 per cent malaria due to local exposure.

The county rates per 100,000 population for malaria acquired outside the United States are shown on the accompanying map (Fig. 3).

DISCUSSION

The basic data from which this report has been compiled can not be regarded as infallible. In many counties the certification of malaria as the primary cause of death is subject to challenge, although in most cases malaria was probably contributory to death. A source of greater statistical error is the designation of malaria as a cause of morbidity on the grounds of clinical examination without laboratory confirmation.

At a time when an increasing number of laboratory personnel are becoming available in the accurate diagnosis of malaria parasites in blood films, greater and greater difficulty is being experienced in discovering the parasites in routine and survey examinations. For example, in some states where malaria has been hyperendemic for a century or more the current examination of as many as one thousand or more survey films may reveal only a fraction of one per cent infection. This does not necessarily justify the conclusion that malaria is on the point of becoming extinct in such localities but it certainly suggests that the disease is on a continued decline, or in formerly hyperendemic area has changed from an acute to a mildly chronic, essentially quiescent form. Furthermore, when clinical reports unconfirmed by competent film examination indicate widespread malariousness in an area, the epidemiologist of the state in question should have authority to challenge the reports and,

if no factual basis for the frequency is discovered, to prevent such reports from being entered in the permanent records of the state department of health.

The comments which have been made reflect seriously on the public health intelligence of each political subdivision of a state and of the state as a whole. In spite of the inconvenience occasioned by a lack of statistics it might be better for a state department of health to admit the unreliability of the data or refuse to release them rather than to have the bureau of vital statistics furnish the figures without qualification and then have the epidemiologist, the malariologist or the director of the public health laboratory of the state refer to them as untrustworthy. The analyst who utilizes data based on different qualities of unreliability is seriously penalized in attempting to provide an overall picture of the disease throughout the country and from year to year in a particular geographical area of the country.

It seems likely that in such an infection as malaria, which is rapidly declining in areas of previous hyperendemicity and has reached the point where laboratory confirmation is becoming exceedingly laborious and difficult, the statistical evaluation of the disease will become comparably difficult and may even be impractical.

SUMMARY

1. Malaria mortality data for the year 1946, obtained from Federal sources, have been analyzed. Only 341 deaths attributable to malaria were reported for the year. This represents a continued year-by-year decline in the number of malaria deaths since 1933, the last peak year, when 4678 deaths were reported.

2. Malaria morbidity data for the year 1946, obtained from Federal sources and in many instances rechecked against state records, provide for the first time a breakdown of data by counties for malaria acquired in the Continental United States and outside the Continental United States. Approximately 50 per cent of the counties reported one or more cases of malaria. Of these approximately half, primarily in the southeastern states and adjacent areas of Texas and Oklahoma, reported a predominance of native malaria. Although there was some overlapping, approximately one half, primarily in the northern, essentially nonmalarious part of the United States, reported almost exclusively cases of malaria acquired outside the country.

3. An analysis of the data from which this report has been compiled indicates that the sources of information are not equally reliable. It is suggested that state departments of health should critically examine the raw data received from their political sub-divisions and not release information which their epidemiologists regard as inaccurate or questionable.

REFERENCES

Faust, E. C. 1932. The 1930 status of malaria in the Southern United States. So. Med. Jour., **25**, 544–549.

Faust, E. C., Scott, J. A., and McDaniel, G. E. 1947. Malaria mortality and morbidity in the United States for the year 1945. Jo. Nat'l Malaria Soc., **6**(3), 184–191.

VICTOR HAAS NAMED DIRECTOR OF MICROBIOLOGICAL INSTITUTE

Dr. Victor H. Haas has been designated as Director of the newly created Microbiological Institute in the National Institutes of Health, Bethesda, Maryland. This Institute, established by the Surgeon General of the Public Health Service on November 1, 1948, will include the Laboratory of Infectious Diseases, the Laboratory of Tropical Diseases and the Laboratory of Biologics Control as well as the Rocky Mountain Laboratory at Hamilton, Montana.

Dr. Haas entered the Public Health Service in 1931 following graduation from the University of Cincinnati, College of Medicine, and was commissioned Assistant Surgeon the following year.

With the exception of an assignment to U. S. Marine Hospital, Stapleton, New York and a two year detail to the Army, his service career has been spent in medical research.

During two previous assignments to the National Institute of Health, he conducted laboratory and field research on streptococcal diseases, lymphocytic chorio-meningitis and poliomyelitis as well as epidemiological investigations in connection with the 1933 outbreak of St. Louis encephalitis.

For four years he was engaged in both field and laboratory research on plague, his work being instrumental in tracing the spread of plague among indigenous rodents to regions not known to be infected.

In 1941 he was appointed by the Surgeon General as Chief of the Medical Commission to the Yunnan-Burma railway which planned and directed programs of malaria control, general sanitation, and medical care for a labor force of more than 100,000 Chinese laborers operating on a right-of-way of some 300 miles of mountains and jungles.

Upon the fall of Burma in April of 1942 he was detailed by the Public Health Service to the U. S. Army, China-Burma-India Theatre. His first assignment was as malaria control officer and epidemiologist on the General Staff, Service of Supply for that theatre. For his later services as Surgeon for the Base Section constructing the Stilwell Road from Asam to China, he was awarded the Legion of Merit upon his release from the Army in December 1943.

Since that time he has served as Chief of the Subdivision on Malaria, Division of Tropical Diseases and as Officer in Charge of Malaria Investigations, National Institutes of Health, Memphis, Tennessee.

He is the author of numerous scientific publications and is a member of the American Medical Association, American Public Health Association, American Society of Tropical Medicine, National Malaria Society and Association of Military Surgeons of the United States.

A COMPARISON OF THE RESIDUAL EFFECTIVENESS OF CERTAIN INSECTICIDES AGAINST *ANOPHELES QUADRIMACULATUS*

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The introduction of DDT has stimulated wide search for other organic chemicals which might prove to be superior to DDT as residual insecticides. Three new organics, chlorinated camphene (Parker, et al., 1947), chlordane (Kearns, et al., 1945), and DDD were used in the present study in comparison with DDT to determine their action as residual insecticides when applied to the interior surfaces of houses for the control of *Anopheles quadrimaculatus*.

Field experiments using an insect release method adapted from that described by Tarzwell and Stierli (1945) were conducted in test rooms during the summer of 1946. The rooms used were located east of Savannah, Georgia in an abandoned section of a war-housing project. These rooms were uniform in construction and measurements. Each was 10 feet square with the ceiling 8 feet 4 inches high and contained one door and a double window. All rooms were entirely vacant except for a crude corner closet consisting of a wood side and two high shelves. The walls were painted cement block, the ceiling was unpainted composition wallboard and the floor was smooth cement.

PROCEDURE

The insecticides were applied to the walls and ceilings of these rooms at the rate of 200 mg. of the active ingredient per square foot. All spraying was performed by one operator using a cylindrical, 4-gallon, air-pressure hand sprayer equipped with a straight wand and nozzle. This equipment delivered a 50° fan-shaped spray at the rate of 0.2 gallon per minute at a pressure of 40 pounds per square inch. A pressure gauge was attached to the sprayer for greater accuracy of spray application.

After spraying, the floor of each room was covered with paper in order to facilitate the collection of knocked-down mosquitoes. No tests were made in any room until at least two weeks after application, when it was considered that the solvent and emulsifier had dissipated.

Tests were made by releasing 150 to 600 or more insectary-reared, 3- to 4-day-old *A. quadrimaculatus* mosquitoes in each treated room and observing the rate of knockdown at 20-minute intervals over a period of four hours. The knocked-down mosquitoes were collected with an aspirator, counted and recorded according to sex, although only the female counts were considered in the comparisons studied herein. As a check of the condition of the insects used in each release, a small number (usually 20–30) were transferred from each rearing cage into a small, cylindrical, screen wire holding cage before each release. These mosquitoes were then held in an ad-

jacent untreated room and observed periodically during the test for possible weakness. It was found unnecessary to repeat any tests because of manifest weakness of the test insects.

The residual toxicity of the following insecticides was compared: (1) DDT (2,2-bis(parachlorophenyl)-1,1,1-trichloroethane), (2) Chlordane (1,2,4,5 6,7,8,8-octochloro-4,7 methano-3a,4,7,7a-tetrahydroindane), also known under the trade name "*1068*," (3) chlorinated camphene containing 67–69 percent chlorine and known under the trade names of *Toxaphene*[1] or "*3956*," and (4) DDD (2,2-bis(parachlorophenyl)-1,1-dichloroethane). In comparing these toxicants the following eight formulations were used, each containing 5 percent of the active ingredient: (1) DDT xylene emulsion from 35-percent-DDT concentrate, (2) DDT suspension from 90-percent-DDT water-wettable powder, (3) DDT suspension from 50-percent-DDT water-wettable powder, (4) chlordane xylene emulsion from 35-percent-chlordane xylene concentrate, (5) chlordane kerosene emulsion from 50-percent-chlordane kerosene concentrate, (6) chlorinated camphene-xylene emulsion from 35-percent chlorinated camphene-xylene concentrate, (7) chlorinated camphene suspension from 25-percent-chlorinated camphene water-wettable powder, and (8) DDD xylene emulsion from 35-percent-DDD xylene concentrate. In all cases where an emulsifier was needed, *Triton X-100*[2] was used.

Each of the above formulations was applied at the rate of 200 mg. of the active ingredient per square foot, and three rooms were sprayed with each combination. The 5 per cent DDT xylene emulsion is adopted herein as a standard for comparison with the other formulations.

DISCUSSION OF SPRAY CHARACTERISTICS

Certain impressions regarding the application characteristics of the several sprays may be noted.

The characteristic odor of DDT xylene emulsions is well known and is not considered objectionable, especially since it disappears as soon as the sprayed surface has dried. The DDT water-wettable mixtures were essentially odorless. The DDD xylene emulsion left a moderately strong but not lingering odor, which was likened to the smell of old straw. Chlordane, in both emulsions, had a very noticeable odor which was somewhat reminiscent of cedar oil and which could be readily detected in the sprayed rooms for more than two months. The chlorinated camphene xylene emulsion left no strong characteristic odor except that the xylene odor seemed to be tinged with a slightly piney scent. The chlorinated camphene water-wettable suspension was practically odorless.

None of these formulations caused unusual discomfort for the spray operator with the exception of the chlordane kerosene emulsion which, probably due to the solvent used, was rather irritating to the eyes.

All the emulsions were very readily sprayable with the equipment used. The suspensions gave some difficulty, showing tendencies to clog the nozzle in proportion to the amount of the inactive agent in the original powders. The 90 per cent DDT

[1] *Toxaphene* is a product of the Hercules Powder Co., Wilmington, Del.

[2] *Triton X-100* is a product of the Rohm and Haas Co., Philadelphia, Penn.

water-wettable suspension sprayed more readily than the 50 per cent DDT water-wettable powder while the most difficulty was experienced with the 25 per cent chlorinated camphene water-wettable powder.

All the water-wettable formulations formed unstable suspensions, consequently the spray container had to be agitated continuously during application.

Residues left by the above emulsions were invisible or nearly so, while the water-wettables left a white deposit which would be objectionable to most householders.

RESULTS

Table 1 shows the comparative results of tests made with these insecticides at periods up to nearly seven months after application. It was not possible to make all tests at exactly comparable intervals, chiefly due to unavoidable fluctuations in the available supply of test mosquitoes. It is believed, nevertheless, that the data herein presented show the relative residual qualities of the insecticide fromulations involved.

DDT, from the standpoint of residual effectiveness, was clearly superior to other insecticides as formulated for these tests. The results obtained with the 5 per cent DDT xylene emulsion and the 5 per cent DDT suspension from the 90 per cent DDT water-wettable powder were notably similar. The rate of knock-down, except in the third test, in which the DDT water-wettable residue acted more slowly, was approximately the same throughout. At the end of the fourth test, the four-hour knock-down was 1.4 per cent better in favor of the suspension-treated rooms; however, neither this nor the differences in residue age can be considered significant. The treatment with 5 per cent DDT suspension from the 50 per cent DDT water-wettable powder was markedly slower in speed of knock-down than the spray made from the 90 per cent powder.

The 50 per cent water-wettable deposit was somewhat inferior to the 90 per cent water-wettable in rapidity of knock-down up to 79 days after application, and became nearly 20 per cent less efficient in residual action after 176 days, in terms of four-hour knock-down. This difference in residual value between the DDT water-wettable suspensions may be due in some degree to a masking effect by the inert ingredients which are present in proportionately greater amounts in sprays from 50 per cent DDT powders than in sprays of equal concentration from 90 per cent DDT powders.

DDD, which was tested at residue ages up to 192 days, killed mosquitoes much more slowly than DDT, but more rapidly than chlorinated camphene, and in the fourth test, somewhat more rapidly than chlordane residues of comparable ages. The four-hour knock-down figures for each test were more erratic than DDT and therefore fail to show deterioration of residue as clearly. Nevertheless, it seems apparent from these data that DDD is a less effective toxicant against *A. quadrimaculatus* mosquito adults than is DDT.

Tests with chlordane showed it to be slightly more effective when applied in a kerosene emulsion than in a xylene emulsion. The chlordane kerosene emulsion showed a distinctly more rapid initial knock-down than the chlordane xylene emulsion, and even after 200 days seemed to have retained slightly more activity. Chlor-

TABLE 1

Cumulative percentages of knock-down of adult female A. quadrimaculatus *mosquitoes after exposures of one to four hours in rooms with 200 mg. per square foot deposits of various insecticides using indicated formulations and with ages ranging from 19 to 201 days. Results are derived from weighted averages* of data from three rooms treated with each formulation.*

INSECTICIDE	FORMULATION	TEST NO.	MEAN AGE OF RESIDUE	PER CENT KNOCKED DOWN AT HOURLY INTERVALS				PER CENT REMAINING AFTER 4 HRS.
				1 hr.	2 hr.	3 hr.	4 hr.	
			days					
DDT	Xylene emulsion	1	23	45.6	90.5	97.9	100	0
		2	50	36.2	85.8	96.2	99.1	.6
		3	103	29.7	75.2	91.9	98.1	1.4
		4	191	9.9	51.3	83.4	94.3	5.6
DDT	90% water-wettable	1	19	19.1	85.2	97.0	99.6	.2
		2	65	42.3	83.2	96.1	99.6	.3
		3	124	2.6	42.8	80.4	96.9	2.6
		4	179	13.0	57.0	86.4	95.7	4.2
DDT	50% water-wettable	1	26	12.1	71.6	93.0	99.0	.9
		2	79	13.0	50.7	80.7	93.4	6.6
		3	127	1.9	13.4	45.4	71.8	27.9
		4	176	8.6	30.6	54.9	77.2	22.4
DDD	Xylene emulsion	1	17	11.1	54.3	76.8	86.7	12.7
		2	45	3.3	24.0	52.2	68.3	31.8
		3	95	6.9	34.6	62.1	82.6	17.4
		4	192	.6	14.4	43.9	74.6	25.3
Chlordane	Xylene emulsion	1	23	7.6	62.6	97.0	100	0
		2	54	6.8	92.2	97.6	100	0
		3	109	2.0	19.0	62.7	94.2	5.6
		4	201	3.1	6.2	42.3	67.9	32.0
Chlordane	Kerosene emulsion	1	28	20.6	94.6	99.4	100	0
		2	54	12.6	83.9	98.2	99.9	.1
		3	110	1.0	30.7	64.2	97.5	2.1
		4	200	.9	6.8	46.2	71.8	28.1
Chlorinated camphene	Xylene emulsion	1	20	.9	12.8	57.0	78.1	21.7
		2	78	2.1	6.0	21.1	48.1	51.8
		3	131	.2	.8	3.9	17.8	82.1
		4	167	.5	2.3	6.7	17.6	82.2
Chlorinated camphene	25% water-wettable	1	23	.8	5.1	37.7	71.7	28.0
		2	87	1.4	11.0	38.6	74.0	25.9
		3	132	1.3	5.4	33.2	64.1	35.9
		4	170	2.3	3.7	5.0	6.8	93.2

* Percentage knock-down in each room weighted in proportion to the square root of the number of mosquitoes released in that room, using the formula $\frac{W_1X_1 + W_2X_2 + W_3X_3}{W_1 + W_2 + W_3}$ where "W" is the square root of the number of mosquitoes used in each room test and "X" is the percent knock-down.

dane compared very favorably with DDT in residual action up to a period somewhat exceeding three months. At an age of 200 days, the xylene and kerosene formulations of chlordane produced respectively 26 per cent and 22 per cent less knock-down in four hours than the DDT xylene emulsion at 191 days. The apparently significant difference in the residual quality of the two insecticides is that DDT loses effectiveness slower, having retained a high level of activity over a period greater than the duration of these tests. Chlordane, on the other hand, though highly effective for more than three months, demonstrated a rather rapid decline in toxicity during the next three months. These findings are essentially in accord with those of Fay, et al. (1947), and Cutkomp (1947).

Chlorinated camphene, in each of the formulations applied, proved to be much slower in knocking down mosquitoes than the other insecticides tested. The chlorinated camphene xylene emulsion showed an initial knock-down activity superior to that of chlorinated camphene water-wettable residues applied at an equal rate. However, the deterioration of the residue from the emulsion was much more rapid during the first 130 days than was the deterioration of the residue from the suspension. Tests of each at the age of 167–170 days showed that neither produced a knockdown as much as 20 per cent during a four-hour exposure with the suspension apparently less effective than the emulsion residue. This phenomenon, in which chlorinated camphene emulsion deteriorated more rapidly, but not as much as the chlorinated camphene suspension, is not explained. It coincides approximately with the results of laboratory tests by Fay, et al. (1947) up to a residue age of 20 weeks.

SUMMARY

Comparative tests were made by releasing insectary-reared *Anopheles quadrimaculatus* mosquitoes in rooms treated with 200 mg. residues of eight spray combinations as follows: (1) 5 per cent DDT xylene emulsion, (2) 5 per cent DDT suspension from 90 per cent DDT water-wettable powder, (3) 5 per cent DDT suspension from 50 per cent DDT water-wettable powder, (4) 5 per cent DDD xylene emulsion, (5) 5 per cent chlordane xylene emulsion, (6) 5 per cent chlordane kerosene emulsion, (7) 5 per cent chlorinated camphene xylene emulsion, and (8) 5 per cent chlorinated camphene suspension from 25 per cent chlorinated camphene water-wettable powder. The DDT-xylene emulsion appeared to be superior to the other insecticides tested, both in speed of knock-down and in residual qualities. DDT applied in suspension from a 90 per cent water-wettable powder was more effective than sprays formulated from a 50 per cent water-wettable powder and approximately equal to the DDT xylene emulsion. Chlordane compared favorably with DDT in initial knockdown but displayed a shorter residual life. It was slightly more effective when sprayed as a kerosene emulsion than as a xylene emulsion. DDD acted more slowly than either DDT or chlordane, but seemed superior to chlorinated camphene, which was very slow acting and seemed to have a comparatively short residual life.

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REFERENCES

(1) Parker, W. Leroy, and Beacher, John H.: Toxaphene a chlorinated hydrocarbon with insecticidal properties. Univ. Delaware Agri. Exp. Sta., Bull. 264, Tech. **36**: 27 p. (1947).

(2) Kearns, C. W., Ingle, Lester, and Metcalf, Robert L.: A new chlorinated hydrocarbon insecticide. Jour. Econ. Ent. 38 (6): 661–668 (1945).

(3) Tarzwell, Clarence M., and Stierli, Harry: The evaluation of DDT residual sprays for the control of Anopheline mosquitoes in dwellings. Pub. Health Rep., Suppl. No. **186**: 35–48 (1945).

(4) Fay, R. W., Cole, E. L., and Buckner, A. J.: Comparative residual effectiveness of organic insecticides against houseflies and malaria mosquitoes. Jour. Econ. Ent. **40** (5): 635–640 (1947).

(5) Cutkomp, Laurence K.: Residual sprays to control *Anopheles quadrimaculatus*. Jour. Econ. Ent. 40 (3): 328–333 (1947).

SOME FACTORS INFLUENCING THE RESIDUAL EFFECTIVENESS OF DDT AND CHLORDANE IN ANOPHELINE MOSQUITO CONTROL

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There are many factors, such as temperature, humidity, types of surfaces treated, and exposure to which treated surfaces are subjected, influencing the residual effectiveness of DDT and other insecticidal chemicals, over which it is impossible to exercise any degree of effective control in practical field application. There are others such as dosage, time of treatment, thoroughness of application, and frequency of retreatment which can be controlled. It is with these latter factors that this discussion is concerned.

The repeated use of DDT (2,2-bis(parachlorophenyl)-1,1,1-trichloroethane) during the past three years on the Extended Malaria Control Program of the various state health departments in cooperation with the U. S. P. H. S., has created operational problems as to the most desirable dosage at which future treatments of previously treated premises should be applied and the frequency with which such retreatments should be made. Previous work by McCauley et al. (1946a) indicated that of the newer insecticides being offered in competition to DDT for use in homes, chlordane more nearly approached DDT in effectiveness as a residual spray than any other. To more fully evaluate the relative toxicity of DDT and chlordane against *Anopheles quadrimaculatus* mosquitoes and to develop information which might prove useful in planning future residual treatments of homes for malaria control, several series of experiments were initiated early in 1947 at the Savannah laboratory of the Communicable Disease Center.

PROCEDURE

In these tests, unoccupied houses of concrete block construction were used, the inner walls of which had been painted with water paint several years previously. Treatments were applied only in the bedrooms of each housing unit, as these rooms were of uniform size and construction. The rooms measured 10 ft. x 10 ft. x 8 ft. 4 in. and each had two windows and one door. The ceilings were constructed of plasterboard and the floors were made of concrete.

All treatments were applied with a conventional hand air-pressure sprayer, equip-

[1] Several members of the Technical Development Division assisted in these studies. Special thanks are due Senior Scientist S. W. Simmons, who gave valuable advice and assistance in the work, and to Senior Assistant Sanitarian (R) Richard W. Fay for his aid and suggestions in planning the tests and for his supplying the laboratory-reared mosquitoes used in the test releases. Dr. William M. Upholt also assisted in planning the tests. Biological Aide Charles W. Ivey made many of the test releases and Mrs. Rosetta D. Edwards made the calculations used in the tables.

ped with a 5002 Spraying Systems nozzle, which produced a flat, fan-shaped spray pattern. Each treatment was applied as a single coverage of the surface area, the different dosages being obtained by changing the strength of the emulsions used. Each application was begun with an initial air pressure of 45 psi in the spray can, which decreased to approximately 30 psi at the end of the treatment. Since the same equipment was used in making all applications and the rooms were of uniform size and construction, the quantity of spray applied in each room could be kept constant by timing the spraying operation. Before treatment, the window shades were drawn to prevent fogging of the windows, and all large cracks and crevices in the walls and ceiling were covered with masking tape. All interior surfaces of the room were then covered uniformly with the spray. Following treatment, the floors were covered with paper to facilitate recovery of the knocked-down test insects.

One series of previously untreated rooms was treated with DDT at single application dosages of 200, 300, 400, and 800 mg. of DDT per sq. ft. of treated surface area, and in one case with two applications of 200 mg. of DDT per sq. ft. per treatment, the second treatment being applied approximately three months after the first treatment. Similar dosages of chlordane were used, except that no rooms were treated with the 300 mg. per sq. ft. dosage. The 300 mg. DDT treatment was included in the experiment to secure data for possible use in the Extended Malaria Control Program of the Communicable Disease Center and was considered only in comparison with other DDT applications. A group of three rooms was treated with each dosage of each insecticide to be tested, making a total of 27 rooms used in the experiment.

To test the effect of the presence of furniture, a set of simulated furniture was constructed for each of the rooms, except those sprayed with 300 mg. of DDT per sq. ft. Each set of furniture, which was made of unpainted wood and cardboard, consisted of a dresser, a chest of drawers, a bed, a table, a chair and two pictures. The backs and undersides of this furniture were treated at the same dosages and at the same time that the walls and ceilings of the rooms were treated. A few sets of furniture also were constructed and left untreated. Each time these rooms were tested, releases of test mosquitoes as described below were made on successive days in the rooms with treated furniture, with untreated furniture, and with all furniture removed.

To secure data on the toxicity of DDT and chlordane applied on previously treated surfaces, nine rooms treated with DDT and six with chlordane in 1946, were retreated in the spring of 1947. The 1946 treatments had all been applied at the rate of 200 mg. of insecticide per sq. ft. Two each of the rooms treated in 1946 with DDT were resprayed in the spring of 1947 with dosages of 50, 100, and 200 mg., and one with 400 mg. of DDT per sq. ft. Of the rooms treated in 1946 with chlordane, two each were resprayed in 1947 with 100 and 200 mg., and one with 400 mg. of chlordane per sq. ft. Two of the rooms treated in 1946 with DDT and one treated with chlordane were not resprayed but observations of the effectiveness of the old treatments were continued.

All treatments referred to above were applied as xylene-*Triton X-155*[2] emulsions, prepared from 35 per cent (wt./vol.) concentrates of the insecticides. The DDT

[2] An emulsifier produced by Rohm and Haas Co., Philadelphia, Pa.

concentrates were composed of 35 gms. DDT, 2 ml. *Triton X-155* and Xylene to make 100 ml. The chlordane concentrates were the same except that they contained 4 per cent *Triton X-155*.

The effectivness of the various treatments was evaluated by the anopheline mosquito release procedure described by McCauley et al. (1946b), except that the interval at which the knocked-down insects were recovered was varied somewhat. In these release tests, approximately 500 insectary-reared adult *A. quadrimaculatus* mosquitoes were released in each room. Examinations were made at 30-minute intervals for a period of four hours after release, and the knocked-down mosquitoes were picked up with aspirators and counted. The number of each sex recovered was recorded, but only the female mosquitoes were considered in evaluating the effectiveness of the treatments. The number of minutes required to obtain a knock-down of 50 per cent and 95 per cent of the mosquitoes released in the rooms was computed by interpolation from the counts made at the 30-minute intervals. A small sample from each lot of mosquitoes used in each test was held for observation to determine normal mortality. Four test releases were made in each room at approximately 1½-month intervals during the six-month period following treatment.

RESULTS AND CONCLUSIONS

The results of the release tests in the rooms treated for the first time in 1947 with DDT and chlordane are given in table 1. These data indicate that DDT is superior to chlordane in long-term residual effectiveness. At the 200 mg. per sq. ft. dosage, DDT surpassed chordane in toxicity. At the 400 mg. dosage, the two chemicals were very nearly equal, while at the 800 mg. dosage, chlordane was better than DDT during the first part of the test, but failed to hold its advantage throughout. The early effectiveness of the higher dosages of chlordane is believed to have been produced by its strong fumigant action.

In considering only the DDT applications, there was little if any consistent and clear-cut difference in the residual effectiveness of any of the single applications regardless of dosage employed. During the last half of the test period, a second 200 mg. per sq. ft. treatment applied three months after the first treatment gave generally better results than the single application, although its margin of superiority over the single 400 and 800 mg. treatments was very slight, if any.

The effect of the presence of furniture in unoccupied treated rooms is shown by the data given in table 2, which compare the effectiveness of treated rooms without furniture, with treated furniture, and with untreated furniture. In the rooms containing furniture, the knocked-down mosquitoes were collected at 30-minute intervals during the first release test, which was made 1½ months after treatment. It was noted that the activity of the observer entering the rooms at such frequent intervals and moving around and under the furniture to pick up the knocked-down mosquitoes, tended to disturb the mosquitoes which were resting on the furniture. Many of the mosquitoes which had been resting on untreated surfaces of the furniture when disturbed by this activity of the observer, came to rest on treated surfaces. To reduce this disturbance to a minimum and more nearly simulate actual field conditions, observations during the remainder of the tests were made only at the end of the second

TABLE 1

The average time in minutes required to obtain 50 per cent and 95 per cent knock-downs of insectary-reared adult female A. quadrimaculatus *mosquitoes after exposure to residual deposits of various dosages of DDT and chlordane emulsions in unoccupied unfurnished rooms. Results are weighted averages of test data from three rooms each.*

Insecticide		DDT	DDT	DDT	DDT	DDT	Chlordane	Chlordane	Chlordane	Chlordane
Dosage applied per sq. ft.		200 mg.	200 mg. + 200 mg.*	300 mg.	400 mg.	800 mg.	200 mg.	200 mg. + 200 mg.*	400 mg.	800 mg.
Preparation		5% emulsion	5% emulsion	7½% emulsion	10% emulsion	20% emulsion	5% emulsion	5% emulsion	10% emulsion	20% emulsion
	%									
Minutes required for knock-down 1½ months after treatment†	50	62	59	50	53	52	58	85	45	47
	95	104	97	111	92	94	109	112	84	75
Minutes required for knock-down 3 months after treatment†	50	73	93	83	64	70	116	99	75	55
	95	142	163	166	110	116	164	147	109	89
Minutes required for knock-down 4½ months after treatment†	50	90	72	92	78	68	111	76	82	55
	95	153	123	184	150	115	157	124	115	86
Minutes required for knock-down 6 months after treatment†	50	106	85	94	95	95	219+	191	114	140
	95	194+	156	193	153	165	240+	214+	165	189

* Second treatment of 200 mg. applied immediately following the 3-month check of the original treatment of these rooms.

† Interpolated from counts made at 30-minute intervals.

TABLE 2

Cumulative percentages of knock-down of insectary-reared adult female A. quadrimaculatus *mosquitoes at the end of two-hour and four-hour periods of exposure to residual deposits of various dosages of DDT in unoccupied rooms with no furniture, treated furniture, and untreated furniture. Results are weighted averages of test data from three rooms each.*

Insecticide and dosage applied per sq. ft.		DDT, 200 mg.			DDT, 200 mg. + 200 mg.*			DDT, 400 mg.			DDT, 800 mg.		
Preparation		5% emulsion			5% emulsion			10% emulsion			20% emulsion		
Room furnishings		None	Treated	Untreated	None	Treated	Untreated	None	Treated	Untreated	None	Treated	Untreated
	%KD												
1½ mos. after treatment	In 2 hr.	98.3	98.1	86.3	99.4	98.5	80.9	96.6	100	96.5	99.6	99.4	88.7
	In 4 hr.	100	100	99.7	100	100	99.7	100	100	100	100	100	100
3 months after treatment	In 2 hr.	87.3	66.1	40.4	77.1	68.1	46.0	95.9	75.1	38.3	97.2	72.9	57.7
	In 4 hr.	99.9	99.6	98.5	99.4	100	98.4	100	98.3	95.4	100	100	100
4½ mos. after treatment	In 2 hr.	79.7	76.9	59.5	92.7	59.1	43.8	86.8	53.9	42.4	97.4	85.6	57.4
	In 4 hr.	99.5	100	96.9	100	100	99.9	99.3	99.5	98.6	100	100	99.5
6 months after treatment	In 2 hr.	63.9	44.2	26.7	81.9	41.8	37.7	78.8	55.9	28.4	76.4	63.2	32.9
	In 4 hr.	94.9	96.6	86.6	100	100	89.4	100	99.7	86.5	99.6	99.2	87.4

* Second treatment applied immediately following the 3-month check of the original treatment in these rooms.

and fourth hours. The effect of this change in procedure undoubtedly is responsible for the great difference between the results of the first test and those of the subsequent tests.

The data in table 2 verify the findings of McCauley et al. (1946b) that the effectiveness of a residual treatment of DDT varied directly with the degree of completeness with which the available resting surfaces were treated. In every instance, regardless of dosage applied, the rooms without furniture gave the best results, followed by rooms with treated furniture and untreated furniture, respectively. A significant finding in these tests was that throughout the six-month test period, the effectiveness in the rooms treated at the single 200 mg. per sq. ft. dosage and containing furniture treated at the same rate, was better than that in the rooms with untreated furniture at any other dosage tested, including those receiving a second treatment of 200 mg. per sq. ft. applied approximately three months after the first application. This increased effectiveness was apparent chiefly in the speed of knock-down, for after four hours the knock-down was so near 100 per cent in all cases that little difference could be noted during the first $4\frac{1}{2}$ months.

The procedure most commonly followed in house spraying with DDT for the control of adult anopheline mosquitoes in the United States, has employed two treatments of only the walls and ceilings at the rate of 200 mg. of DDT per sq. ft. per treatment, the applications being made in the early spring and midsummer at an interval of approximately three months. It is recognized that the condition under which the tests discussed above were conducted, did not completely duplicate actual field conditions, in that the mosquitoes were confined in the experimental rooms during the test releases, and some additional untreated resting surfaces would be available to the mosquitoes in the form of clothing and other miscellaneous articles in occupied houses. Nevertheless, the increased effectiveness in the rooms containing treated furniture in these tests was so striking that the implications are that by treating the backs and undersides of furniture as well as the walls and ceilings, a single treatment per season may give as good control as the two treatments per season usually employed, where the furniture was not treated. Treatments at dosage rates higher than 200 mg. per sq. ft. appear to be unnecessary if the furniture is treated. The backs and undersides of all furniture are unfinished, and the furniture is pulled back from the walls during usual spraying procedures, thereby exposing the backs in such a manner that they could be treated with very little additional effort. With the exercise of reasonable care and discretion by the spray crews, the addition of furniture treatment should become a practical and effective modification of the present house spraying techniques in future malaria control programs.

Table 3 gives the results of the retreatments in 1947 of rooms originally treated in 1946. These data further accentuate the superiority of DDT over chlordane with regard to long-term residual toxicity. The rooms treated with DDT in 1946 at the rate of 200 mg. per sq. ft. continued to produce appreciable knock-down of mosquitoes throughout 1947 without retreatment. Retreatments of some of these rooms at dosage rates of 50, 100, 200, and 400 mg. of DDT per sq. ft. restored their effectiveness nearly to their original toxicity but failed to produce any evidence of accumulative insecticidal effectiveness. In general, the effectiveness of the retreatments

increased as the dosages were increased, the best results being obtained with the 400 mg. retreatment.

The data in tables 1 and 3 indicate that retreatment in 1947 of rooms treated in 1946 failed to give as good results as did original 1947 treatments at comparable dosages. The differences are not great, but are evident in most of the comparisons. On the other hand, when considered for the entire 6-month experimental period, the group of rooms (table 1) which received a second treatment in 1947 approximately three months following the first treatment, gave the same general results during the second three months as during the first three months. In the case of both types of retreat-

TABLE 3

Average time in minutes required to obtain 50 per cent and 95 per cent knock-downs of insectary-reared adult female A. quadrimaculatus *mosquitoes after exposure in unoccupied unfurnished rooms treated with residual sprays in 1946 and retreated in the spring of 1947 approximately one year after the original treatment*

Insecticide		DDT	DDT	DDT	DDT	DDT	CHLORDANE	CHLORDANE	CHLORDANE	CHLORDANE
Original dosage—mg./sq. ft.		200	200	200	200	200	200	200	200	200
Retreatment dosage—mg./sq. ft.		None	50	100	200	400	None	100	200	400
	%									
Minutes required for knock-down	50	144	84	84	89	66	195	105	106	78
1½ months after retreatments*	95	+	178	171	157	128	+	164	146	119
Minutes required for knock-down	50	129	110	106	83	117	171	124	113	87
3 months after retreatments*	95	+	220	208	156	193	+	182	178	124
Minutes required for knock-down	50	191+	108	121	102	71	+	127	114	73
4½ months after retreatments*	95	+	198	197	186	133	+	176	162	112
Minutes required for knock-down	50	143	121	107	98	72	+	197	+	164
6 months after retreatments*	95	+	193	212	174	139	+	+	+	+

+ Over 240 minutes required for 95 per cent knock-down.

* Interpolated from counts made at 30-minute intervals.

ments, there was a definite improvement in effectiveness following retreatment, as compared to similarly treated rooms which were not retreated.

Numerous reports were received in 1947 from the field regarding the failure of retreatments to give as good results as initial treatments. Insofar as a single retreatment at the rate of 200 mg. per sq. ft. and over is concerned, the data in tables 1 and 3 do not indicate any appreciable reduction in effectiveness as compared to the original treatment. What effect additional retreatments may have on subsequent toxicity remains to be determined.

SUMMARY

1. In a study of some of the factors influencing the residual effectiveness of field applications of DDT and chlordane for anopheline mosquito control, rooms in un-

occupied concrete block houses were treated with dosages ranging from 200 to 800 mg. of insecticide per sq. ft. of treated surface. Single applications and one retreatment of previously treated surfaces were tested. Insectary-reared adult *A. quadrimaculatus* mosquitoes were used in making the tests to evaluate the effectiveness of the residual treatments.

2. At dosages within the limits of practical application, DDT proved to be superior to chlordane with respect to long-term residual effectiveness.

3. In view of the experimental variation between sets of observations, it is concluded that there is little if any consistent and clear-cut difference in the residual effectiveness of 200, 300, 400, and 800 mg. concentrations of DDT as employed, especially when compared on the basis of 50 per cent knock-down. A second application of 200 mg. three months after the initial one definitely reduced the average knockdown time as observed one and one-half months thereafter. At the end of six months, the retreated series showed little advantage over the single applications as regards knockdown time.

4. Untreated furniture in a treated room significantly decreased the effectiveness of a spray application.

5. Throughout the six-month test period, the effectiveness in rooms treated at the rate of 200 mg. per sq. ft. and containing treated furniture was superior to that in the rooms treated with any other dosage but containing untreated furniture, in that more mosquitoes were generally knocked down in a shorter period of time. After four hours, however, the knockdown was so near one hundred per cent that the difference was negligible until six months after treatment.

6. By retreatment with a second 200 mg. per sq. ft. dosage, surfaces previously treated with DDT were restored to almost the same effectiveness as followed the original treatment, but failed to show any evidence of accumulative insecticidal effectiveness.

REFERENCES

(1) McCauley, Robert H., Fay, R. W., and Simmons, S. W. 1946a. A comparison of the residual effectiveness of certain insecticides against *Anopheles quadrimaculatus*. Public Health Report (In press).

(2) McCauley, Robert H., Fay, R. W., and Simmons, S. W. 1946b. The importance of coverage in DDT residual house spraying for the control of *Anopheles quadrimaculatus* mosquitoes. Public Health Report **63**: 401–407 (1948).

WHO MALARIA COMMITTEE REPORTS ON SECOND SESSION

The Second Session of the Expert Committee on Malaria of the World Health Organization Interim Commission took place in Washington, D. C., May 19–25, 1948, at the Pan American Sanitary Bureau. This Committee comprises: Dr. Arnoldo Gabaldon, Venezuela, Chairman; Major General Sir Gordon Covell, U.K.; Dr. Paul F. Russell, U.S.A.; Med. General Marcel Vaucel, France; Dr. D. K. Viswanathan, India and the Secretary, Dr. Emilio Pampana, WHO.

The report of this second session is interesting, readable but too lengthy to reprint in full. However, the gist of the report is contained in the conclusions and recommendations as follows:

1. *WHO Malaria Policy.* With reference to the International programme for malaria control on a world wide basis, the *Committee* deems it advisable to *recommend:*

(a) that WHO be prepared to assist governments on request, through regional organizations, when established, in setting up on a permanent basis malaria control services suited to local needs, and that these services should be of adequate size, staffed by adequately paid and adequately trained personnel;

(b) that the main objective of the services mentioned above should be effective control of malaria at the lowest feasible cost and adapted to the limitations of the budgetary capacity of each government;

(c) that the services of individual experts and operational-demonstration teams be made available to give adequate advice and practical assistance to governments in order to foster the development of local and national malaria control programmes;

(d) that the services mentioned in the preceding paragraph be constituted on a temporary basis; and that in reference to teams, their function should be to carry out malaria economic surveys of the area concerned, to put in practice a control programme based on these surveys and to assess results and costs in terms of malaria, general health and economics; that such control demonstrations should be planned on a scale which could be expanded by the respective governments within the limits of their budgetary capacity. Plans should be made for each team to remain in the area until the control programme, which will be primarily carried out by residual insecticide methods, is established and the preliminary results assessed. This service should not be of less than two years' duration;

(e) that when operational-demonstration teams be sent to a country, a condition to obtain WHO cooperation should be, that the governments be required to appoint local officers to understudy each member of the team from outside sources;

(f) that whilst it may be necessary for the Secretariat to assist in procuring the services of individual experts, the constitution of the teams and their arrangements for their dispatch to the area to be protected should be the responsibility of the regional organizations as soon as they are established;

(g) that all necessary equipment and adequate motor transportation for the operational-demonstration teams be included in the initial budgets for such teams;

(h) that three of these teams be formed as early as possible and that they should be allocated on request by governments to selected areas in (i) Central Africa, (ii) Southeast Asia, and (iii) the Tropical Americas;

(i) the assistance on request to schools of malariology now in operation by providing expert lecturers who would participate in the teaching programme, or by other help as indicated;

(j) the assistance in setting up courses in malariology in regions now provided with such facilities, i.e. in Central Africa and in Southeast Asia. For example, a revival of the International Malaria Courses formerly held in Singapore under the auspices of the Health Organization of the League of Nations might be considered as meeting certain immediate needs in Southeast Asia. Similar courses might be instituted in Central Africa;

(k) the provision of fellowships and travel grants for training in malariology;

(l) the provision of teams or individuals as required for training purposes in the application of particular techniques of malaria control;

(m) the dissemination of reports and manuals dealing with malaria control measures;

(n) that in malaria courses or malaria control demonstrations aided by WHO, due attention be given to education of the public on this subject, and that even more important is the vital need to acquaint administration officers and engineers of all branches with the basic principles of malariology;

(o) that WHO, through its Secretariat, attempt to collect and to distribute to official public health organizations such material on malaria propaganda as is available from all over the world;

(p) that attention be given to the feasibility of building up in each regional organization of WHO a lending service of cinema films, film-strips, lantern slides, and other educational material on the subject of malaria and its control;

(q) an appropriate modification of article 14 of the "Draft Regulations Applicable to Expert Committees and their Sub-Committees" (WHO.IC/140, Rev. 1, 7 February 1948) to provide for the "corresponding members" to work on a regional basis within the regional organizations, when they will be established;

(r) that the Secretariat, through the regional organizations, should carry out relevant studies of national, and in larger countries, of state or provincial budgets, paying due regard to the proportion of each budget spent on health activities in general and on malaria control in particular.

2. *Agriculture and malaria.* With reference to this problem, *the Committee recommends:*

(a) that when the time comes for selecting demonstration areas the simplest procedure may be for WHO to select a number of areas and for FAO to advise the prospects of increased production in the areas named, the final choice made by appropriate consultations;

(b) that the two Secretariats (WHO and FAO) should collaborate in examining

the problem of selecting areas on the basis of (1) feasibility of effective malaria control, and (2) potentiality as regards increased food production, and prepare a joint report for further consideration.

3. *Insecticides.* The Committee considers that the application of DDT as an adulticide is the method of choice in a widespread attack on rural malaria, and that by its use a significant reduction of morbidity may be effected in the majority, if not in all malarious countries. Further investigation is required to assess the efficacy of this insecticide against *A. gambiae* in Africa.

In order to stimulate the use and production of DDT and to render it available to all malarious contries, *the Committee recommends:*

(a) that the question of regional production of DDT, solvents and wetting and emulsifying agents and the waiving of customs duty on these insecticides in non-manufacturing countries be referred to the appropriate Bodies of the Economic and Social Council of the United Nations;

(b) that an *Expert Sub-Committee on Insecticides* be set up to specify international standards for insecticides and their formulation, to stimulate the development of standard spraying equipment on a regional basis, and to deal with all other questions relating to the proper use of insecticides;

(c) that research on insecticides be encouraged with special emphasis on determining their stability, effective particle size, duration of residual action in different surfaces, optimum formulations, toxicity to beneficial insects and wild life, and the possibility of development of resistant strains of mosquitoes;

(d) that literature be interchanged on spraying techniques, formulations equipment, organizational details, costs and results of spraying operations.

4. *Chemotherapeutics in malaria control*

(a) *The Committee* agrees that the primary consideration in the communal control of malaria is interruption of transmission at the mosquito level, and *recommends* that measures so directed should be given priority by health authorities whereever possible: agrees on the value of drug administration (apart from clinical indications) under special circumstances which are briefly:

(i) the immediate clinical control of epidemic malaria;

(ii) the clinical control, under restricted conditions, of endemic malaria;

(iii) the suppression and suppressive cure of existing human infection.

(b) The Committee is not prepared at the present time to give firm recommendations for the use and dosage of the new compounds but has summarized information in Section VI which may serve as a provisional guide.

5. *Research. The Committee recommends:*

(a) that coordinated hospital and field trials on malaria chemotherapy and chemoprophylaxis be carried out under the auspices of WHO in different countries;

(b) that an experimental project of species eradication in the absence of natural barriers be carried out in Central Africa under the auspices of WHO.

6. *Quarantine against reimportation of anophelines. The Committe recommends:*

(a) that the World Health Assembly take steps to put into effect immediately, with the reservations expressed in Section VIII of the present report, the measures envisaged in the Draft International Agreement concerning steps

for the prevention of importation of malaria vectors into regions cleared of anophelines (Sardinia). (Doc. WHO.IC/Mal/22);

(b) that the local authority be empowered to carry out immediate disinsectization of any ship which does not possess a valid disinsectization certificates;

(c) that whatever regulations be enforced regarding disinsectization of seacraft or aircraft, rigid anti-mosquito sanitation should, as far as practicable, be maintained within mosquito flight range of ports and airports of the country to be protected, so that no imported mosquitoes will be able to survive;

(d) that apart from the specific case of Sardinia, a draft International Sanitary Regulation to prevent importation of anophelines, and applicable to all areas requiring such protection, be studied by the Expert Committee on International Epidemic Control jointly with the Expert Committee on Malaria.

STUDIES IN HUMAN MALARIA

XX. The Intramuscular Administration of Chloroquine[1]

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(Received for publication 15 October 1948)

Chloroquine (SN 7618), or 7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline, is rapidly becoming established as an effective antimalarial drug, capable of alleviating infections caused by *Plasmodium falciparum*, *P. vivax* or *P. malariae* (Loeb *et al.*, 1946; Most *et al.*, 1946; Gordon *et al.*, 1947; Young and Eyles, 1948; Berliner *et al.*, 1948). It appeared to us of practical interest to determine whether the drug could be given parenterally for the initiation of therapy in situations where oral dosage was not feasible.

In preliminary trials in *rhesus* monkeys, Schmidt (1947) found that chloroquine hydrochloride, in 4.5 per cent aqueous solution given intramuscularly, was well tolerated in dosages up to and including 20 mgm. of chloroquine (base) per kgm. of body weight. Dosages of 40 mgm. per kgm. were fatal to 3 of 4 monkeys. Plasma concentrations of chloroquine reached high levels 15 min. after injection, e.g., after 5 mgm. per kgm.: 922 μgm. per liter; after 10 mgm. per kgm.: 1,682 μgm. per liter; and after 20 mgm. per kgm.: 3,556 μgm. per liter. The acute toxicity was slightly greater than that of intramuscularly administered quinacrine (atabrine): 40 mgm. of the latter drug per kgm. produced marked toxemia and 60 mgm. per kgm. was promptly fatal. Histological examination of the sites of injection revealed little evidence of local tissue damage after chloroquine, in contrast to marked damage after comparable dosages of quinacrine.

In the clinical studies reported below, chloroquine hydrochloride was given intramuscularly in 16 instances for the therapy of experimental sporozoite-induced *vivax* malaria in prisoner volunteers.

MATERIALS AND METHODS

All of the attacks treated were either primary or relapse episodes during Chesson strain *vivax* infections in young white male volunteers. The technique of infection by the bites of 10 infected female *Anopheles quadrimaculatus* mosquitoes and the

[1] Papers I through XIX of this series are appearing in the American Journal of Hygiene.

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[3] Now in the Department of Medicine, University of Chicago, Chicago, Ill.

[4] With the professional and technical assistance of S. A. Nurse Officer (R) Virginia D. Hines, Chemist Virginia Duff-White and Medical Technicians Julia R. Wess and Elizabeth G. Guinn.

Grateful acknowledgment is extended to the officials of the Bureau of Prisons, Department of Justice, for making these studies possible; to the Warden and the custodial staff of the Federal Correctional Institution, Seagoville, Texas, for actively facilitating the conduct of the project; and to the inmate volunteers for their willing participation.

routine procedures carried out during 18 months of close observation have been previously described (Coatney *et al.*, 1948).

Chloroquine was given as the hydrochloride in sterile unbuffered aqueous solution,[5] containing 45 mgm. of the salt (equivalent to 40 mgm. of the base) per milliliter. The pH of the solution was 5.6. Dosages, unless otherwise stated, are expressed in terms of the base.

Treatment in each attack was begun on the third day of patent parasitemia if the oral temperature had reached 102°F. by 8 a.m. of that day; if first fever occurred between 8 a.m. of the third day and 8 a.m. of the fourth day, treatment was begun on the fourth day. Therapy was never deferred beyond the fifth day of patent parasitemia, regardless of the temperature record.

Blood samples for parasite counts and chloroquine estimations were taken before and at intervals of 15 min., 30 min., 1 hr., 4 hrs. and 24 hrs. after injections. Blood smears for the detection of parasites were made daily until at least 3 negative smears had been secured, then twice weekly for the detection of relapses. Pulse and blood pressure determinations were made at frequent intervals during the first hour after injections.

Plasma concentrations of chloroquine were estimated photofluorometrically after ultraviolet irradiation, using the method of Brodie *et al.* (1947).

TEST AND RESULTS

Three dosage regimens of chloroquine were used, as follows:

I. Single injection of 200 mgm.

II. Two injections of 200 mgm. each, given 4 hrs. apart.

III. Single injection of 300 mgm.

Five primary attacks of malaria, in volunteers S-84, S-85, S-86, S-87 and S-88, were utilized for the trial of regimen I. The first relapses in the same 5 subjects were terminated with regimen II. Regimen III was used in 6 cases, the second attacks of volunteers S-101, S-103 and S-105 and the third attacks of volunteers S-102, S-104 and S-106. When calculated on the basis of the patients' body weights, the doses of chloroquine ranged from 2.5 to 5.5 mgm. per kgm. (table 1).

Injections were made in the gluteal muscles. After due precautions had been taken against intravenous entry, the drug was introduced as rapidly as possible. There were no subsequent signs or symptoms which would indicate either local or systemic reaction. Pulse and blood pressure did not change significantly in any of the volunteers.

The plasma concentrations of chloroquine during the post-injection periods are shown in table 1. Although there were great differences in the levels attained by different volunteers, it will be seen that in all but 4 instances the highest concentrations observed were those at 15 min. after injection. All subjects achieved levels high in the therapeutic range during the first hour. The highest concentration observed in any subject was 480 μgm. per liter.

Clinical response was rapid in all cases. Fever and patent parasitemia were promptly ended (see table 2). Relapses, expected after a non-curative drug, occurred in all but one instance, parasites reappearing 16 to 19 days after the single

[5] Supplied in sealed ampules of 10 milliliters each by Winthrop-Stearns Inc., New York, N. Y.

TABLE 1

Plasma concentrations of chloroquine in human volunteers with vivax *malaria after the intramuscular administration of chloroquine hydrochloride*

VOLUNTEER	CHLOROQUINE DOSE		CHLOROQUINE CONCENTRATIONS (μ GM. PER LITER) IN PLASMA DRAWN AT THE FOLLOWING INTERVALS (IN HOURS) AFTER FIRST INJECTION								
	Mgm. base	Mgm. base per kgm.	¼	½	1	4	4½	4¾	5	8	24
S-84	200	2.9	79	71	56	36	—	—	—	—	7
S-85	200	2.7	108	53	14	7	—	—	—	—	7
S-86	200	2.6	161	77	43	12	—	—	—	—	10
S-87	200	3.3	70	80	53	14	—	—	—	—	5
S-88	200	2.5	57	30	19	12	—	—	—	—	5
S-84	200 × 2*	2.9 × 2	65	108	58	18*	120	106	137	46	26
S-85	200 × 2*	2.7 × 2	77	48	34	12*	55	50	48	48	14
S-86	200 × 2*	2.7 × 2	108	89	106	19*	139	79	96	43	36
S-87	200 × 2*	3.3 × 2	163	137	65	22*	127	125	110	43	31
S-88	200 × 2*	2.5 × 2	86	63	43	22*	88	64	48	67	34
S-101	300	4.4	226	130	53	0	—	—	—	—	0
S-102	300	5.5	65	89	48	48	—	—	—	—	19
S-103	300	5.5	480	245	132	48	—	—	—	—	19
S-104	300	3.9	135	101	70	46	—	—	—	—	24
S-105	300	4.0	240	96	48	24	—	—	—	—	9
S-106	300	4.4	79	70	43	12	—	—	—	—	7

* Second injection 4 hrs. after first, immediately after drawing blood sample.

TABLE 2

Clinical and parasitological response in 16 attacks of vivax *malaria terminated with intramuscularly administered chloroquine hydrochloride*

VOLUNTEER	ATTACK	CHLOROQUINE DOSE	PARASITES PER 5,000 RBC ON DAY OF THERAPY	RESULTS OF THERAPY		
		Mgm. base		Days to negative smear	Days until afebrile (<101°F.)	Days to repatency
S-84	1st	200	150	2	2	32*
S-85	1st	200	990	2	2	16
S-86	1st	200	70	1	1	31*
S-87	1st	200	840	2	2	18
S-88	1st	200	3,150	2	2	19
S-84	2nd	200 × 2†	740	2	1	42
S-85	2nd	200 × 2†	1,650	2	1	27
S-86	2nd	200 × 2†	380	2	1	33
S-87	2nd	200 × 2†	1,290	2	2	42
S-88	2nd	200 × 2†	6,960	2	2	34
S-101	2nd	300	240	1	—	24
S-102	3rd	300	3,330	3	1	35
S-103	2nd	300	440	2	—	115
S-104	3rd	300	290	1	—	47
S-105	2nd	300	520	1	—	30
S-106	3rd	300	2,970	3	2	—

* Interim treatment delayed relapse in S-84 and in S-86.

† Second injection 4 hrs. after first.

200 mgm. dosage, 27 to 42 days after the double 200 mgm. dosage and 24 to 115 days after the 300 mgm. dosage.

DISCUSSION

These exploratory studies, in which chloroquine hydrochloride was given intramuscularly, indicate the feasibility of such parenteral administration for the patient unable to retain medication given by mouth. This conclusion is strengthened by the experiences of Terry and Spicknall (1948) at the U. S. Marine Hospital, Baltimore, Md. These workers have now utilized intramuscularly administered chloroquine to begin treatment in 8 patients with naturally acquired *falciparum* malaria and have observed satisfactory clearance of parasites and no harmful reactions.

None of the patients included in the foregoing studies was critically ill, so that it is impossible to conclude from the data that chloroquine given intramuscularly would prove rapidly efficacious in overwhelming infections where there is peripheral vascular stasis. The slow intravenous administration of chloroquine deserves study in such situations, due regard being given to the possible depressive action of high concentrations upon the central nervous system. It is not unlikely that cautious intravenous administration, in large saline infusions, protracted over 3 or 4 hours, as practiced by Machella *et al.* (1947) with quinacrine and SN 6911, would prove effective. In the absence of such studies, the intramuscular route is recommended for routine parenteral administration. It is to be strongly emphasized that the absorption of chloroquine from the alimentary tract is usually so rapid that parenteral administration should be resorted to only in exceptional cases.

The regimens in our studies were limited to single or double doses of drug in order to define clearly the effects of intramuscular administration. In actual practice, one would recommend that oral therapy be begun as soon as possible so as to complete a full course of approximately 1.5 grams of chloroquine within 3 days. Such a regimen would minimize the chance of *falciparum* recrudescence and would delay the reappearance of *P. vivax*.

SUMMARY

1. Chloroquine (SN 7618) hydrochloride, in 4.5 per cent aqueous solution, was given intramuscularly in doses of 200 or 300 mgm. of base (2.5 to 5.5 mgm. per kgm.) in 16 cases of experimental Chesson strain *vivax* malaria.

2. No toxic reactions occurred and the antimalarial action was comparable to that from oral medication.

3. It is suggested that where oral administration of chloroquine is not feasible, therapy can be initiated by the intramuscular injection of chloroquine (200 mgm. of base for adults), with repetition after 4 hours if necessary. Treatment by mouth, to complete a course of approximately 1.5 grams of base in 3 days, should be started as soon as practicable.

REFERENCES

Berliner, R. W., Earle, D. P., Jr., Taggart, J. V., Zubrod, C. G., Welch, W. J., Conan, N. J., Bauman, E., Scudder, S. T., and Shannon, J. A. 1948. Studies on the chemotherapy of the human malarias. VI. The physiological disposition, antimalarial activity, and toxicity of several derivatives of 4-aminoquinoline. Jour. Clin. Invest., **27**: (No. 3, Pt. 2): 98–107.

Brodie, B. B., Udenfriend, S., Dill, W., and Chenkin, T. 1947. The estimation of basic organic compounds in biological material. III. Estimation by conversion to fluorescent compounds. Jour. Biol. Chem., **168:** 319–325.

Coatney, G. R., Cooper, W. C., and Ruhe, D. S. 1948. Studies in human malaria. VI. The organization of a program for testing potential antimalarial drugs in prisoner volunteers. Amer. Jour. Hyg., **47:** 113–119.

Gordon, H. H., Dieuaide, F. R., Marble, A., Christianson, H. B., and Dahl, L. K. 1947. Treatment of *Plasmodium vivax* malaria of foreign origin; a comparison of various drugs. Arch. Int. Med., **79:** 365–380.

Loeb, R. F., et al. 1946. Activity of a new antimalarial agent, chloroquine (SN 7618). Statement approved by the Board for Coordination of Malarial Studies. Jour. Amer. Med. Assoc., **130:** 1069–1070.

Machella, T. E., Kimmelman, L. J., and Lewis, R. A. 1947. The intravenous administration of atabrine in *falciparum* malaria. Bull. U. S. Army Med. Dept., **7:** 1009–1021.

Most, H., London, I. M., Kane, C. A., Lavietes, P. H., Schroeder, E. F., and Hayman, J. M., Jr. 1946. Chloroquine for treatment of acute attacks of *vivax* malaria. Jour. Amer. Med. Assoc., **131:** 963–967.

Schmidt, L. H. 1947. Personal communication.

Terry, L. L., and Spicknall, C. G. 1948. Personal communication.

Young, M. D., and Eyles, D. E. 1948. The efficacy of chloroquine, quinacrine, quinine and totaquine in the treatment of *Plasmodium malariae* infections (quartan malaria). Amer. Jour. Trop. Med., **28:** 23–28.

STUDIES IN HUMAN MALARIA

XXI. The Cure of St. Elizabeth Strain *Vivax* Malaria by Pentaquine-Quinine, Administered during Acute Attacks or during Latency

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Concurrently administered pentaquine[4] and quinine have been shown to be curative of *vivax* malaria under a variety of experimental and natural conditions (Loeb, 1946; Monk, 1948; Alving *et al.*, 1948; Straus and Gennis, 1948; Coggeshall *et al.*, 1948). All published studies, however describe therapy given in the customary manner during acute malarial attacks, and it appeared of theoretical interest to determine whether curative treatment would be equally effective in preventing relapses when given during the latent phases of the disease. If relapses result from persistent non-circulating parasites, it would not seem that the eradication of such parasites need bear any relation to the presence or absence of circulating erythrocytic parasites at the time of treatment.

The St. Elizabeth strain of *Plasmodium vivax*, which characteristically produces an infection with a long latent period of 6 to 9 months between the early primary attack and the first relapse, is ideally suited for studying such a point. In the experiments described, pentaquine and quinine in combination were used in the therapy of St. Elizabeth strain *vivax* malaria during primary attacks, during latency and during late relapses.

MATERIALS AND METHODS

The subjects in the study were young white male prisoner volunteers at the Federal Correctional Institution, Seagoville, Texas.[5] The procedures by which they were infected with the St. Elizabeth strain of *P. vivax* by the bites of *Anopheles quadrimaculatus* mosquitoes and the routine followed during their observation over a period of 18 months have been previously described by Coatney *et al.* (1948).

Thick blood smears were examined on all volunteers at least once weekly throughout 18 months of observation. Smears were made daily from day 9 through day 33

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[3] With the professional and technical assistance of S. A. Nurse Officer (R) Virginia D. Hines, Chemist Virginia Duff-White and Medical Technicians Julia R. Wess and Elizabeth G. Guinn.

[4] SN 13,276, or 8-(4-isopropylamino-1-methylbutylamino)-6-methoxyquinoline.

[5] Grateful acknowledgment is extended to the officials of the Bureau of Prisons, Department of Justice, for making these studies possible; to the Warden and the custodial staff of the Federal Correctional Institution, Seagoville, Texas, for actively facilitating the conduct of the project; and to the inmate volunteers for their willing participation.

and twice or thrice weekly for 45 days after each acute attack and during the period 6 to 10 months after exposure, when late relapses were most likely.

While subjects were receiving pentaquine plus quinine, hemoglobin determinations were made daily. Complete blood counts and estimations of methemoglobin, in whole blood, pentaquine in plasma and quinine in plasma were made every other day. In subjects given quinine alone, complete blood counts and plasma quinine estimations were made less frequently.

TABLE 1

Effects of pentaquine and quinine, administered concurrently during early attacks and during latency, on infections caused by the St. Elizabeth strain of Plasmodium vivax

GROUP	VOLUNTEER	SET NO.*	INFECTED MOSQUITOES PER SUBJECT ON DAY 0		ONSET OF PATENCY, EARLY ATTACK	TREATMENT OF PRIMARY ATTACK	TREATMENT DURING LATENCY, FROM DAY 120 THROUGH DAY 133	ONSET OF RELAPSE	TOTAL OBSERVATION PERIOD AFTER EXPOSURE
			No.	Sum of plus					
					day after exposure			*day after exposure*	*days*
A	S-42	1	10	32	12	*Pentaquine,*	None	—	547
	S-43	2	10	34	13	60 mgm. per day,		—	547
	S-44	3	10	37	13	+		—	547
	S-45	4	10	40	12	*Quinine,*		—	547
	S-46	5	10	39	13	2.0 grams per day, for 14 days		—	547
B	S-47	1	10	32	13	*Quinine,*	*Pentaquine,*	—	547
	S-48	2	10	34	12	2.0 grams per day,	60 mgm. per day,	—	547
	S-49	3	10	34	13	for 14 days	+	—	547
	S-50	4	10	40	12		*Quinine,*	—	547
	S-51	5	10	39	14		2.0 grams per day, for 14 days	—	178†
C	S-52	1	10	32	12	*Quinine.*	*Quinine,*	280	302†
	S-53	2	10	34	13	2.0 grams per day,	2.0 grams per day,	257	547
	S-54	3	10	37	14	for 14 days	for 14 days	261	547
	S-55	4	10	40	13			280	547
	S-56	5	10	39	11			199	281†

* Subjects with corresponding set numbers (1 to 5) were bitten by the same mosquitoes.
† Paroled.

When treatment was given during acute attacks, it was started on the third day of patent parasitemia if oral temperature of 102°F. or more had occurred by noon of that day. If first fever occurred between noon of the third day and noon of the fourth day, therapy was started on the fourth day. If no fever had appeared by noon of the fifth day of patent parasitemia, treatment was begun despite the afebrile nature of the attack.

Pentaquine was given as the monophosphate, in tablets which each contained the

equivalent of 5 mgm. of base. Quinine sulfate was given in tablets or capsules, each containing the equivalent of 0.25 gram of quinine base. Dosage regimens, expressed in terms of base content, were as follows:

Pentaquine. 60 mgm. per day (15 mgm. every 6 hrs.) for 14 days.

Quinine. 2.0 grams per day (0.5 gram every 6 hrs.) for 14 days.

Concentrations of pentaquine in plasma were estimated by a method similar to that described by Brodie *et al.* (1947) for pamaquine. Quinine concentrations were estimated by the method of Brodie and Udenfriend (1943).

EXPERIMENT AND RESULT

The 15 men in the study were divided into three groups (A, B and C) of 5 men each. On 19 November 1946 (day 0), they were bitten by 10 *Anopheles quadrimaculatus* mosquitoes infected with the St. Elizabeth strain of *P. vivax*. Each mosquito was allowed to bite one man from group A, group B and group C in turn. Postprandial dissections showed a high density of sporozoites in the mosquitoes' salivary glands (average, 3.6+, on a scale of 0 to 4+); when the total number of pluses was computed for each volunteer, the range was from 32+ to 40+ (table 1).

The men were allowed to develop early primary attacks, which became patent 11 to 14 days after the mosquito bites. On the third to fifth day of patency, treatment was started as follows:

Group A. Concurrent pentaquine and quinine.

Group B. Quinine alone.

Group C. Quinine alone.

The acute attacks were alleviated promptly in all subjects. In the case of the St. Elizabeth strain, the latency following termination of early attacks usually continues until 6 to 10 months after infection, when a wave of late attacks begins. In this experiment the following therapeutic regimens were given during the latent period, beginning at 4 months (120 days) after exposure:

Group A. None.

Group B. Concurrent pentaquine and quinine.

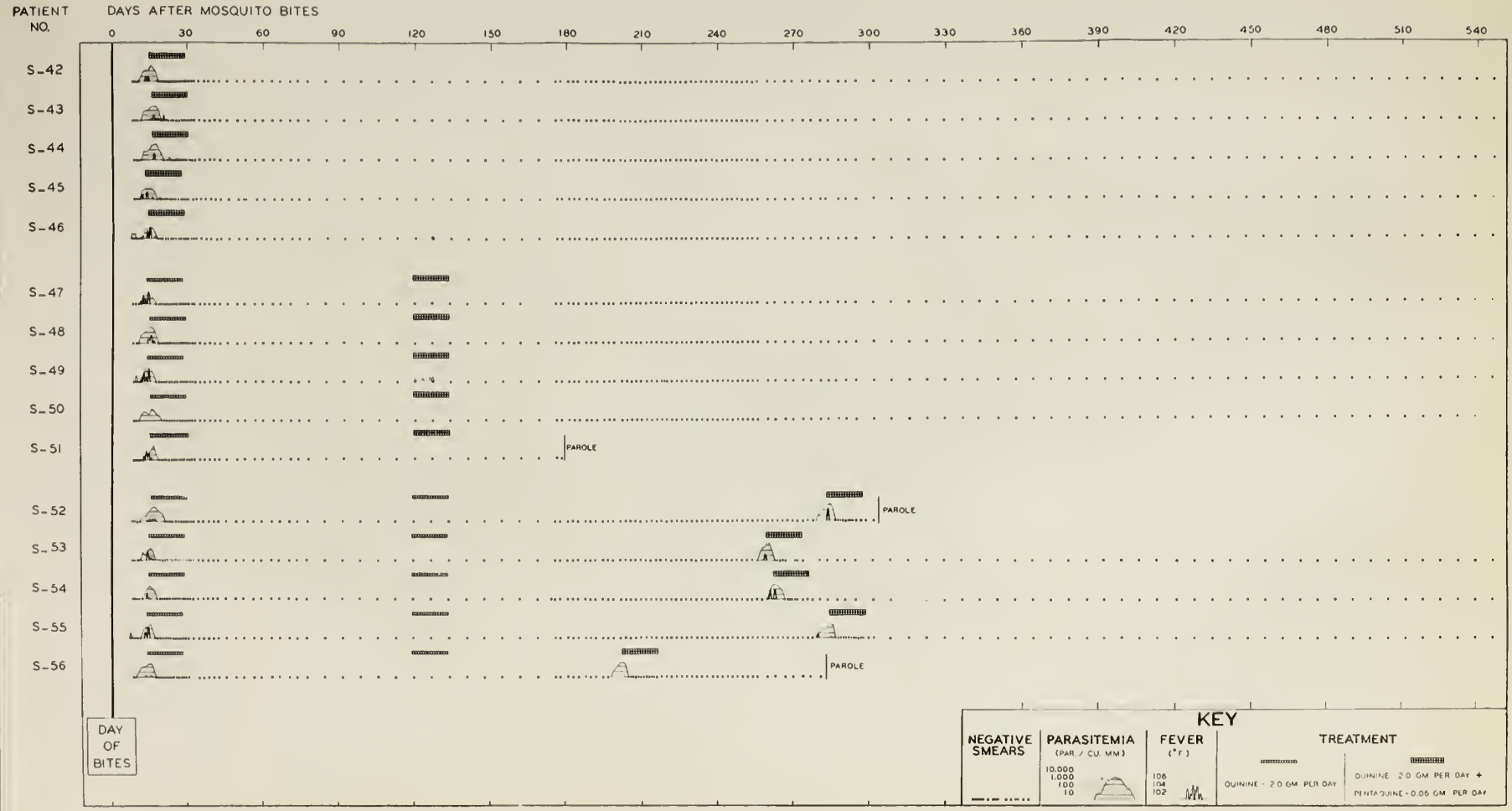
Group C. Quinine alone.

As shown in table 1 and figure 1, the subjects in groups A and B showed no late attacks throughout 18 months of observation while all of the 5 men in group C developed late relapses 199 to 280 days after their original infection, i.e., 171 to 252 days after the end of treatment of their early primary malaria. These late relapses were terminated with combined pentaquine and quinine and no subsequent attacks appeared during a period of observation which extended a full 18 months in 3 of the subjects.

During treatment with concurrent pentaquine and quinine, the mean plasma concentrations of pentaquine for the 15 patients ranged from 36 to 124 μgm. per liter, and the mean plasma concentrations of quinine, from 6.0 to 12.2 mgm. per liter.

FIG. 1. PARASITIC AND CLINICAL ACTIVITY DURING 18 MONTHS OF OBSERVATION IN 15 WHITE MALE VOLUNTEERS WITH SPOROZOITE-INDUCED ST. ELIZABETH STRAIN *Vivax* MALARIA

Volunteers S-42 through S-46 were treated with concurrent pentaquine and quinine during early primary attacks; S-47 through S-51, during latency; and S-52 through S-56, during late relapses


PATIENT NO.
DAYS AFTER MOSQUITO BITES
0
30
60
90
120
150
180
210
240
270
300
330
360
390
420
450
480
510
540
S-42
S-43
S-44
S-45
S-46
S-47
S-48
S-49
S-50
S-51
S-52
S-53
S-54
S-55
S-56
PAROLE
PAROLE
PAROLE
DAY OF BITES
KEY
NEGATIVE SMEARS
PARASITEMIA (PAR. / CU. MM.)
10,000
1,000
100
10
FEVER (°F.)
106
104
102
TREATMENT
QUININE - 2.0 GM. PER DAY
QUININE - 2.0 GM. PER DAY +
PENTAQUINE - 0.06 GM. PER DAY

During the last 5 days of treatment these individuals had mean methemoglobin levels of 2.2 to 9.0 per cent of the total hemoglobin, with the maxima from 3.6 to 10.7 per cent. The subjects receiving quinine alone exhibited mean plasma concentrations of quinine from 5.2 to 9.0 mgm. per liter.

During the 15 courses of combined pentaquine-quinine therapy, there was the following incidence of complaints: abdominal cramps, 14; epigastric tenderness, 9; nausea, 6; anorexia, 4; nausea and vomiting, 3; tinnitus and dizziness, 5; urticaria, 2; fine macular rash, 1; fine papular rash, 1; tremor, 1; blurring of vision, 1. Only one subject had no complaints.

During the 15 courses of quinine alone, the following complaints were recorded: abdominal discomfort, 2; epigastric tenderness, 1; nausea, 3; tinnitus, 3; dizziness, 5; urticaria, 1; fine macular rash, 1; diarrhea, 1.

DISCUSSION

Under the conditions of this experiment, concurrent pentaquine and quinine displayed clearcut curative properties. No volunteer given the combination had subsequent evidence of malaria irrespective of whether treatment was given during early attacks, during latency or during initial late relapses. Quinine given alone did not prevent later attacks, which is in agreement with our previous experience with St. Elizabeth strain *vivax* infections (Coatney *et al.*, 1948a). In a series of over 180 infections, we have observed a relapse incidence of 97 per cent after treatment of early attacks with quinine, quinacrine (atabrine), chloroquine (SN 7618), sontochin (SN 6911), NIH-204 (SN 1796) and SN 5241. The use of these so-called noncurative drugs in initial late attacks has been followed by a relapse incidence of 80 per cent; and if only late attacks treated in the first 10 months after exposure are considered, the relapse incidence has been 92 per cent. Although concurrent pamaquine (plasmochin) and quinine have not been tried in early attacks of the St. Elizabeth strain, when given during late attacks the combination is curative (Ruhe *et al.*, unpublished observations).

The evidence against the existence of circulating parasites during the latent period of St. Elizabeth strain infections is very strong. Repeated blood smear examinations are negative and the transfer of 250 to 300 milliliters of blood to susceptible recipients has not resulted in transmission of infection (Cooper *et al.*, unpublished observations). During this period the subject harboring a latent infection is susceptible to superinfection with erythrocytic parasites of the same strain (Cooper *et al.*, 1947). Action of a drug during the long latent period would, therefore, presumably be confined to action upon the fixed tissue parasites.

The results of these experiments, if they can be translated to other strains of *vivax* malaria, suggest that on the infrequent occasion when such action was desired, pentaquine in combination with quinine could be given as an elective treatment between relapses for the cure of *vivax* malaria.

SUMMARY

Pentaquine (SN 13,276) monophosphate, 60 mgm. of base per day, plus quinine sulfate, 2.0 grams of base per day, given for 14 days prevented subsequent relapses

of St. Elizabeth strain *vivax* malaria when administered during clinical attacks or during latency.

REFERENCES

Alving, A. S., Craige, B., Jr., Jones, R., Jr., Whorton, C. M., Pullman, T. N., and Eichelberger, L. 1948. Pentaquine (SN-13,276), a therapeutic agent effective in reducing the relapse rate in *vivax* malaria. Jour. Clin. Invest., **27** (No. 3, Pt. 2): 25–33.

Brodie, B. B., and Udenfriend, S. 1943. The estimation of quinine in human plasma with a note on the estimation of quinidine. Jour. Pharmacol. & Exper. Therap., **78:** 154–158.

Brodie, B. B., Udenfriend, S., and Taggart, J. V. 1947. The estimation of basic organic compounds in biological material. IV. Estimation by coupling with diazonium salts. Jour. Biol. Chem., **168:** 327–334.

Coatney, G. R., Cooper, W. C., and Ruhe, D. S. 1948. Studies in human malaria. VI. The organization of a program for testing potential antimalarial drugs in prisoner volunteers. Amer. Jour. Hyg., **47:** 113–119.

Coatney, G. R., Cooper, W. C., Ruhe, D. S., Josephson, E. S., Young, M. D., and Burgess, R. W. 1948a. Studies in human malaria. VII. The protective and therapeutic action of quinine sulfate against St. Elizabeth strain *vivax* malaria. Amer. Jour. Hyg., **47:** 120–134.

Coggeshall, L. T., Rice, F. A., and Yount, E. H., Jr. 1948. The cure of recurrent *vivax* malaria and status of immunity thereafter. Proc. Fourth Internat. Congresses on Trop. Med. & Malaria, in press.

Cooper, W. C., Coatney, G. R., and Ruhe, D. S. 1947. Studies in human malaria. V. Homologous strain superinfection during latency in subjects with sporozoite-induced *vivax* malaria (St. Elizabeth strain). Amer. Jour. Hyg., **46:** 141–148.

Loeb, R. F. 1946. Activity of a new antimalarial agent, pentaquine (SN 13,276). Statement approved by the Board for Coordination of Malarial Studies. Jour. Amer. Med. Assoc., **132:** 321–323.

Monk, J. F. 1948. Results of an investigation of the therapeutic action of pentaquin on acute attacks of benign tertian malaria. Trans. Roy. Soc. Trop. Med. & Hyg., **41:** 663–668.

Straus, B., and Gennis, J. 1948. Evaluation of pentaquine as a cure of relapsing *vivax* malaria; a controlled study of ninety-five cases. Bull. New York Acad. Med., 2nd ser., **24:** 395–396.

TOLERABILITY STUDIES OF SOME NEW ANTIMALARIAL DRUGS[1]

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(Received for publication 16 August 1948)

At the request of the Board for the Coordination of Malarial Studies in Washington, D. C., a tolerability test of some of the more promising antimalarial drugs was undertaken by this laboratory. The antimalarial properties of these drugs had been determined previously and further validation was not the object of this study. Experience in the enforcement of "malarial discipline" during World War II had revealed that there were real or fancied objections on the part of some personnel to taking antimalarials for suppression. In the military, especially, the "reputation" of a drug is of considerable consequence. If a new antimalarial becomes associated on a purely psychological basis with nausea and general discomfort, its effectiveness as a prophylactic is, of course, seriously limited. Our purpose was to determine if there were any significant differences in acceptability when these drugs were administered under controlled conditions in dosages considered sufficient for suppression of malarial symptoms. It is hoped that the methods used in the selection of subjects, the experimental design and the development of objective criteria of response will be of some interest and assistance to investigators undertaking similar group studies.

METHODS OF PROCEDURE

Nineteen platoons comprising eleven hundred and twenty-seven men took part in the tolerability tests conducted at the Marine Barracks, Parris Island, South Carolina, from January 10 to March 8, 1946. The subjects were volunteer Marine recruits, between seventeen and twenty years of age, with only two or three weeks prior service in the Marine Corps. All men were undergoing similar phases of training, and were quartered and subsisted in identical fashion. Since the subjects were in "boot training," they were strictly regimented in their daily schedule. Liberty was not permitted. The men taking part in this study were previously screened by a routine, recruit camp, medical examination. This procedure results in a select group, by comparison with the general population, which is relatively uncontaminated with physical or psychiatric disabilities.

Five antimalarial drugs were evaluated. These were SN-6911—0.6 gm.[3], SN-

[1] The opinions and assertions contained in this report are not to be construed as official or as reflecting the view of the Navy Department.

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[3] Dosage of all drugs is expressed in terms of the base.

SN-6911 ($C_{19}H_{28}ClN_3$), 7-chloro-4-(4-diethylamino-1-methylbutylamino)-3-methylquinoline.

SN-7618 ($C_{18}H_{26}ClN_3$), chloroquine.

SN-8137 ($C_{16}H_{22}ClN_3O$), 1-(7-chloro-4-quinolylamino)-3-diethylamino-2-propanol.

SN-11437 ($C_{10}H_9ClN_4O_2S$), N^1-(5-chloro-2-pyrimidyl) metanilamide.

7618—0.3 gm., SN-8137—0.6 gm., SN-11437—2.0 gm., and quinacrine—0.24 gm. Dosage schedules were determined by the Board for the Coordination of Malarial Studies, and were considered to be suppressive when administered once a week. A lactose placebo was included in the test as a control drug. It is recognized that 0.24 gm. of quinacrine given once a week may not necessarily be the most acceptable method of administration. However, since some investigators have reported good suppressive results with this dosage schedule, it was included in this study.

In order to obviate possible group effects, that is, to control interplatoon variability, each platoon (approximately 60 men) was divided at random into six groups of about ten men each. Each subgroup was given one of the six drugs. All men participated on a voluntary basis. The compounds were administered in green-tinted capsules. Placebo capsules were added to those compounds requiring only two drug capsules for their suppressive dose in order to standardize the number of capsules (total of four) administered to each subject. Subjects had no knowledge of the particular compound which they were taking. Each drug was given once a week, over a six-week period, immediately following the noon meal.

After each drug administration, subjects were interviewed concerning the previous week's administration. A combination group and individual interview was used. This procedure was found in preliminary studies on some four hundred men to yield approximately the same incidence of complaints as an individual interview alone and, therefore, was used to facilitate working with a large number of subjects. In the group interview, each platoon was asked several questions such as, "How do you feel", and "Do you have any complaints." Men having any comments responded by raising their hands and falling out of the group. They were then questioned individually as to the nature and time course of their complaints. All complaints were recorded as stated. In addition to the weekly interviews, other items of possible value were obtained. These included a daily record of admissions to the sick list with diagnosis, a record of inoculations, and a listing of the firing scores (M1 rifle) achieved by the men in the different experimental groups.

RESULTS AND DISCUSSION

For reasons of emergency furlough, transfer, guard duty, and similar irrelevant factors, the original population of 1127 was cut to 955 men who had either completed six consecutive weeks of drug administration (892 men) or had, for relevant reasons, refused the drug (63 men). These 955 subjects were divided according to drug as follows:

Drug	*No. of Men*
SN-6911	170
SN-7618	152
SN-8137	165
SN-11437	154
Quinacrine	172
Placebo	142

Criteria for evaluating complaints. Although no absolute criteria for evaluating complaints could be set up, an attempt was made to eliminate at least those symp-

toms which could almost certainly be attributed to some cause other than the experimental drugs. The complaints thus excluded were defined as any which were later found to be part of a well defined symptom-complex of a disease or injury. This eliminated complaints related to strains, sprains, contusions, manifestations of upper respiratory infections, erythematous diseases, food poisonings, reactions to inoculations and localized infections. In addition, all complaints which occurred more than 48 hours after drug administration were excluded. All other complaints were considered to be relevant, even though many of them may not have resulted from the

TABLE 1

Distribution of all complaints according to drug

	SN-6911	SN-7618	SN-8137	SN-11437	QUINACRINE	PLACEBO	TOTAL
Anorexia				1			1
Chest pain						2	2
Chills	1	3	2	4	2	4	16
Constipation				1	4		5
Cramps, abdominal	3	1	7	4	5	3	23
Diarrhea	4	9	7	4	11	5	40
Dizziness			1	2	5		8
Drowsiness	4	2	4	1	1	1	13
Exhaustion	1	1	2			2	6
Flatulence		1		2	1		4
Flush	6	8	8	7	16	6	51
Headache	12	6	4	7	13	5	47
Nausea	9	2	6	1	20		38
Rash	2		2			1	5
Stomach-ache	6		2	2	4	2	16
Syncope			1				1
Vomiting	4	1	3	4	26	2	40
Itch			1				1
Back pain	1						1
Miscellaneous	3	1		1	1		6
Refusal, non-specific		1	1		5	1	8
Total	56	36	51	41	114	34	332

drugs. It was felt that since the placebo control was present, it was wiser to include than to exclude doubtful cases.

Criteria of tolerability. From several possible criteria of tolerability for these experimental drugs, four have been selected. These include:

(a) Refusal to accept the drug after it had been taken at least once.

(b) Complaints attributed to the drug (primarily number of individuals complaining).

(c) Sick bay admissions during the course of drug administrations.

(d) Rifle firing scores achieved during the experiment.

Refusals. A cumulative frequency distribution of individuals refusing to continue the drug may be found in Figure 1. There it will be seen that, although there are no

clear differences between drugs during the early weeks, the differences between numbers of men refusing each drug increase progressively. A statistical check on the significance of the differences between numbers of refusals for each drug, i.e., between the end points on each of the six curves, indicates that both quinacrine and SN-6911

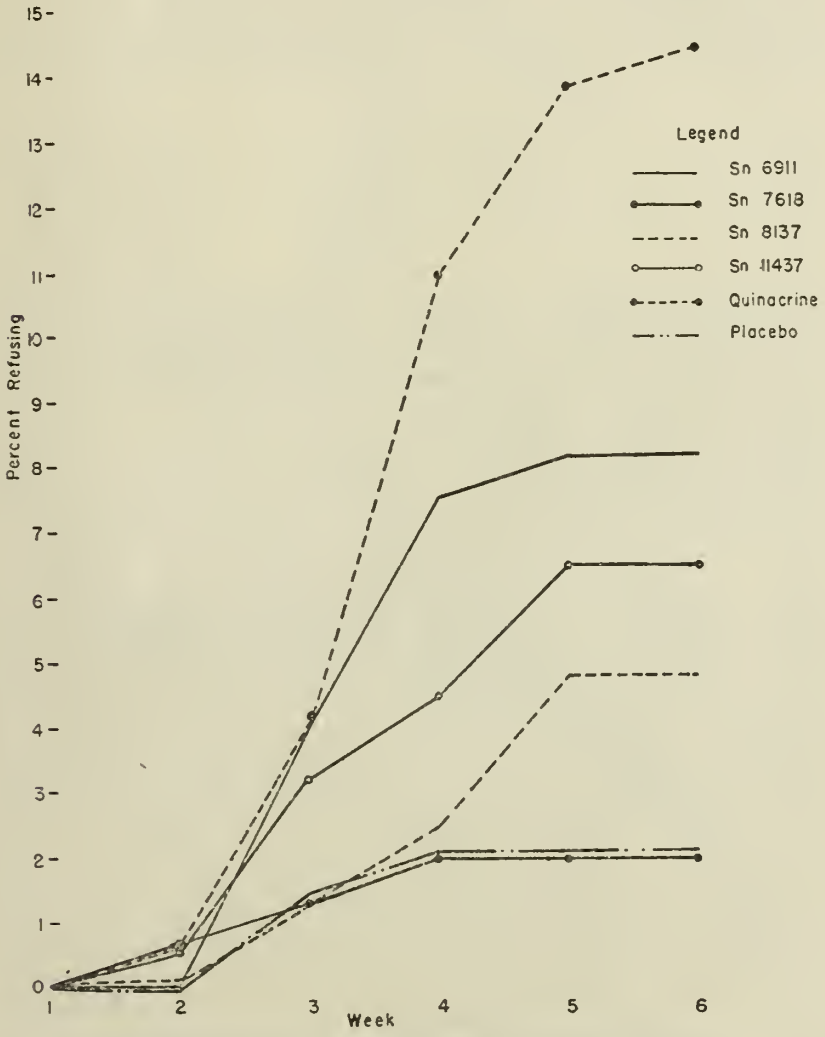

FIG. 1. PERCENTAGE OF REFUSALS BY WEEK—CUMULATIVE

yield significantly more refusals than the placebo ($p. < .01$). With a less rigid criterion, five chances, rather than one chance in a hundred, SN-11437 is also significantly less tolerable than the placebo. The differences of both SN-7618 and SN-8137 from the placebo could have occurred by chance. A further analysis indicates that quinacrine, using a 7 per cent significance level, is significantly less tolerable than its nearest competitor, SN-6911.

Complaints. The second measure of tolerability adopted was number of complaints. Figure 2 presents a cumulative frequency distribution of first complaints including refusals, i.e., the number of men who either complained for the first time, or refused their drug during any given week. It will be noted immediately that the

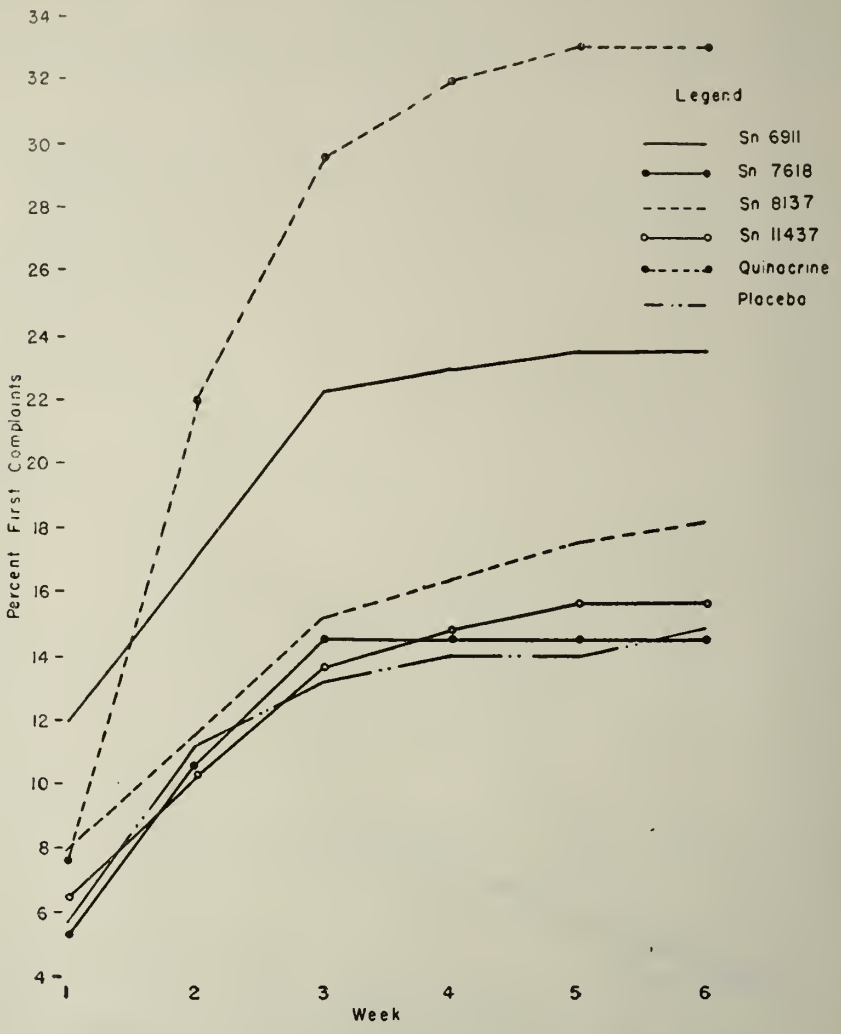

FIG. 2. PERCENTAGE OF FIRST COMPLAINTS BY WEEK—CUMULATIVE

order in which the drugs fall at the end of the sixth week is almost the same for this measure as it was for refusals (except for the reversal of SN-8137 and SN-11437). Once again the significance of the differences between the end points of the curves may be analyzed. Quinacrine is again found to differ from the placebo at better than the 1 per cent level; SN-6911 at better than the 5 per cent level. The difference between each of the other three and the placebo, however, are reasonably likely

to have occurred by chance. The difference between quinacrine and its nearest competitor, SN-6911 is this time significant at better than the 5 per cent level.

Sick bay admissions. An analysis of sick bay admissions was made, including all subjects who had at any time taken the drugs (1127 men) and excluding those diagnoses clearly unrelated to drug administration. The diagnoses thus excluded were injuries and clearly defined symptom complexes. Doubtful diagnoses and those possibly relevant to drug administration were included. The results were as follows:

Drug	*Per cent*
SN-6911	3.1
SN-7618	2.6
SN-8137	1.6
SN-11437	1.6
Quinacrine	3.2
Placebo	1.1

None of the differences between any two of these drugs can be considered significant. However, the two drugs which gave the greatest number of complaints and the greatest number of refusals also yield the greatest number of sick bay admissions.

Rifle scores. The last criterion of tolerability which was evaluated was that of rifle scores. The possibility that some of these drugs might have deleterious effects on motor control had been proposed. It was, therefore, felt that if any such effect existed to a measurable degree it might be ascertained by considering the firing scores of the different drug groups and the controls. No significant differences were found, however, among the average scores of the six groups.

Additional data. In addition to the measures which have been analyzed above, some other evidence, which may be enlightening, is available. In Table 1 is presented a breakdown of type and frequency of complaints for each of the six drugs. The table includes all complaints. It should be pointed out that some individuals ay have registered more than one complaint on any given day. Inspection of the table indicates that for SN-6911 the most common complaint was headache, with nausea a close second; for SN-7618, flushes and diarrhea; for SN-8137, flushes, cramps and diarrhea; for SN-11437, flushes and headaches; for quinacrine, vomiting and nausea; and for the placebo, flushes, headaches and diarrhea. Quinacrine then is the only one whose modal complaints clearly differ from the modal complaints of the placebo. Over 21 per cent of the total complaining population for quinacrine complained of vomiting, while the highest percentage for any of the other drugs was 9.3 per cent (4 men). Similarly, of all the men who vomited, 65 per cent had received quinacrine.

Another phenomenon is described in Figure 3. Here is presented a distribution by weeks of number of complaints for each of the six drugs. This figure differs from Figure 2 in that *all* complaints, whether first or repeated, are included, and also in that the complaints are not cumulative. If one considers only the complaints in these distributions, it will be seen that for every drug there seems to be a gradual falling off in number of complaints as the weeks of the experiment progress. This falling off may be attributed to any one or combination of three factors: first, an actual decrease in number of reactions to the drug, indicating some kind of adaptation; second,

to the knowledge that the experiment is soon to come to an end; or, finally, to the fact that as the weeks go by, the number of men who refuse to take the drug, and therefore drop out of the experiment, has been consistently increasing. Since there is a likelihood that the men who refused would have been most prone to complain had they remained in the experiment, the decrease in complaints might be attributed to the increase in refusals. To check the last factor, a *cumulative* distribution of re-

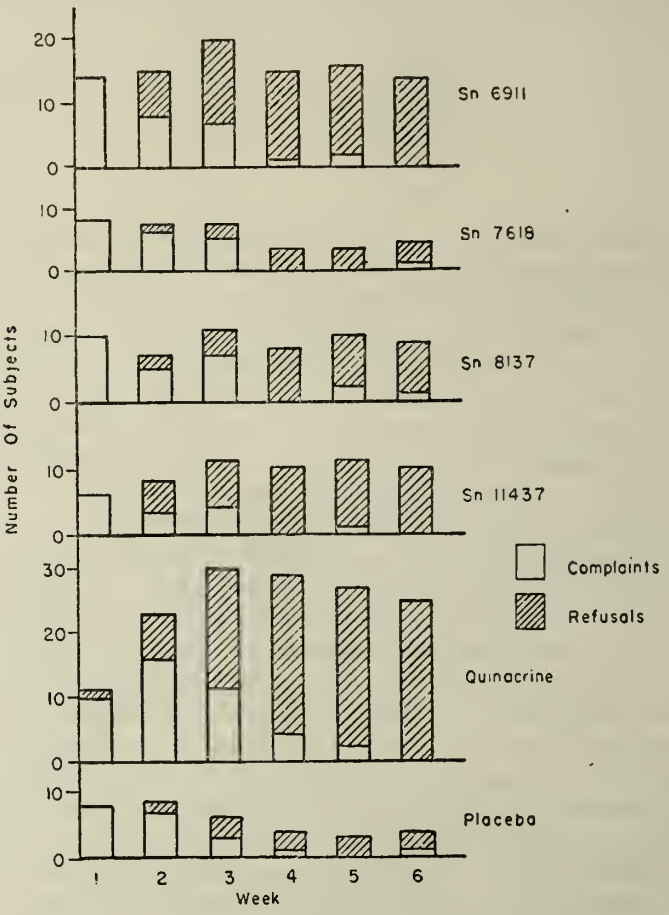

FIG. 3. TOTAL COMPLAINTS PER WEEK (CUMULATIVE DISTRIBUTION OF REFUSALS SUPERIMPOSED)

fusals has been super-imposed upon the distribution of complaints. If for each week all complaints plus the refusals up to that time were to remain at a constant level, one might deduce that the number of complaints was not, in reality, falling off. The apparent falling off may then be attributed primarily to the selectively decreased population resulting from the increasing number of refusals. With the factor of refusals thus held constant, only the placebo and SN-7618 show any clear decrease in number of complaints with time. An attempt was made to compare the frequency

with which individuals complained, but because the number of men who complained more than twice was very small the analysis was discarded.

Refusals and complaints, as well as most of our other results, point to the distinct inferiority of quinacrine and SN-6911 to the other three experimental drugs. On the other hand, none of the drugs can be considered to be definitely superior to all of the others. However, since SN-7618 was the only drug found in most cases to be as tolerable as the placebo, it can reasonably be held to be the best drug in the group from the point of view of acceptability to military personnel.

SUMMARY

1. Four experimental drugs, (SN-6911, 7618, 8137, and 11437), quinacrine, and a lactose placebo were administered on a voluntary basis to 1127 Marine recruits in suppressive doses for six weeks. Subjects were interviewed each week and their complaints analyzed.

2. In terms both of complaints and refusals to continue the drug, quinacrine and SN-6911 were found to be significantly inferior to the other three experimental drugs. SN-7618 yielded fewer complaints and refusals than any of the other compounds. No differences among drugs were discovered from analyses of sick bay admissions.

3. Motor coordination, as reflected by firing scores, was unaffected by the drugs.

4. An apparent falling off in complaints for all drugs during the course of the experiment was found to be attributable, in most part, to a progressively increasing number of refusals. After elimination of the refusals, the remainder of the population showed no cumulative intolerance to the drugs over a period of six weeks.

5. All of the experimental compounds were found to be superior to quinacrine from the point of view of acceptability to a military population. Of the four experimental drugs, SN-6911 was significantly less tolerable than any of the others, while SN-7618 was as acceptable as the lactose placebo.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Drs. R. F. Loeb, E. K. Marshall, Jr., and J. A. Shannon for their interest and advice.

A SEARCH FOR EXOERYTHROCYTIC FORMS IN HUMAN MALARIA BY MEANS OF TISSUE CULTURES OF BONE MARROW[1]

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A program designed to search for exoerythrocytic forms in human malaria was begun in 1946 by making tissue cultures of bone marrow taken in the prepatent period of sporozoite-induced infections (Dubin, 1947). The tissue culture method was used on the chance that if exoerythrocytic forms were present in the specimens they might multiply *in vitro* and thus be easier to detect. The exoerythrocytic forms of *Plasmodium gallinaceum* had been successfully cultivated *in vitro* by Hawking (1945). The bone marrow was used as the explant because it was the only reticulo-endothelial tissue from which samples were readily obtainable. The need for taking samples of spleen and liver as well was recognized, but this material was not available.

Since the onset of this program an unequivocal demonstration of pre-erythrocytic forms of human malaria has been made by Shortt and colleagues (Shortt, Garnham, Covell and Shute, 1948; Shortt and Garnham, 1948). These authors described these forms in a specimen of liver from a patient in the prepatent period of infection with the sporozoites of *P. vivax*. Although the results of the present experiments are essentially negative, it is believed that the work merits a brief description.

METHODS AND MATERIALS

The specimens of bone marrow were obtained by sternal puncture and were heparinized and centrifugalized.[2] Tissue cultures were made from the fatty layer and the buffy coat after these had been clotted with chick embryo juice. The cultures were planted in bottomless Carrel flasks to which coverslips had been cemented (Zuckerman, 1945). The tissue was embedded in human plasma alone or in human plasma reinforced with chicken plasma. Clotting was facilitated by adding a drop of chick embryo juice. The fluid medium consisted of 25 per cent human serum in Tyrode's solution. The cultures were allowed to grow for 4 to 8 days at 37–38 degrees C. They were then fixed with Zenker-formalin fluid, stained with Maximow's stain, and mounted in clarite (Zuckerman, 1945).

In all, 26 successful tissue cultures were made from the bone marrow (Table 1). Of these, 6 were from the prepatent period of infection with *P. falciparum*, 14 from the prepatent period of infection with *P. vivax*, 4 from 1 to 2 weeks after patency in *P. vivax* infections, and 2 during relapse 3 months after initial infection with *P. vivax*.

As a preliminary procedure the technique was tested by cultivating the exoerythro-

[1] This work was supported in part by a grant-in-aid from the Tennessee Valley Authority.

[2] The patients were neurosyphilitics receiving malarial treatment on the Neuropsychiatric Service of the Gailor Memorial Hospital, Memphis. The author is indebted to Drs. T. S. Hill, H. Packer, and Y. T. Wong for the use of the clinical material.

cytic forms of *P. gallinaceum* from infected chick embryos.[3] These forms were successfully grown from spleen, liver, brain and lung under conditions similar to those of the human experiments (Dubin, 1947).

RESULTS

The experiments are summarized in Table 1.

TABLE 1

EXPERIMENT NUMBER	PARASITE	TIME BETWEEN INITIAL INFECTION AND MARROW PUNCTURE	PREPATENT PERIOD	DURATION OF CULTURE	RESULTS
		days	*days*	*days*	
1	*P. vivax*	5	10	4	0
2	*P. vivax*	3 months (relapse)		4	0
3	*P. falciparum*	5	11	4	0
4	*P. falciparum*	5	7	4	0
5	*P. falciparum*	5	9	4	0
6	*P. vivax*	6	11	4	2 questionable forms
7	(Same patient as no. 6)	18		6	0
8	*P. vivax*	6	10	5	0
9	*P. vivax*	6	12	8	0
10	*P. vivax*	6	11	8	0
11	*P. falciparum*	6	11	4	0
12	*P. vivax*	6	9	4	0
13	(Same patient as no. 12)	17		6	0
14	*P. falciparum*	5	7	4	0
15	*P. vivax*	4	10	5	0
16	*P. vivax*	5	11	4	4 questionable forms
17	(Same patient as no. 16)	26		4	0
18	*P. vivax*	4	18	5	0
19	*P. vivax*	4	13	5	0
20	*P. vivax*	4	11	5	0
21	*P. vivax*	3 months (relapse)		6	0
22	*P. falciparum*	4	11	4	0
23	*P. vivax*	4	10	5	0
24	(Same patient as no. 23)	18		4	0
25	*P. vivax*	4	11	5	0
26	*P. vivax*	4	14	4	0

Cell types

A detailed description of the cell types will be given elsewhere. There was multiplication of several types of cells, including a large reticuloendothelial cell which was

[3] This material was supplied through the kindness of Lt. Col. V. H. Haas, Office of Malarial Investigation, United States Public Health Service, Memphis.

probably a stem cell, macrophages, eosinophils, plasma cells, and cells probably belonging to the myelocytic group. In many cultures the macrophages predominated.

Search for parasites

In all, 92 slides were examined; each slide bore a coverslip supporting an area of tissue culture of about 6 cm. square. In one of the early experiments (No. 6) two forms suggestive of exoerythrocytic bodies were noted (Dubin, 1947). In a subsequent experiment, (No. 16) four similar clusters were found in one slide. Each of these consisted of a cluster of about 100 bodies, oval or fusiform, measuring about 2 microns in diameter each, the group having an overall measurement of about 30 microns in diameter. The morphological detail, however, was not quite sharp enough to enable one to identify these structures as parasites. Since the proof in these experiments would depend solely upon morphological criteria and since artefacts could not be categorically excluded, the final decision was to consider these structures as not representing exoerythrocytic forms.

DISCUSSION

To date there has as yet not been any unequivocal demonstration of exoerythrocytic forms in mammalian malaria outside of the liver. In the studies by Shortt and Garnham (1948) with *P. cynomolgi* exoerythrocytic forms were found only in the liver; none were seen in the other tissues including the bone marrow. Huff and Coulston (1947) in a study of human malaria were unable to demonstrate pre-erythrocytic forms in human skin, lymph nodes, veins and muscle after local inoculation of sporozoites of *P. vivax*. Claims for demonstration of exoerythrocytic forms in human bone marrow have not been generally recognized.

The results of the present experiments are being interpreted as negative by this investigator.

SUMMARY

In a search for exoerythrocytic forms in human malaria, tissue cultures were made from the bone marrow of patients with sporozoite-induced infections, both in the prepatent period as well as after development of parasitemia. This was done in *P. falciparum* infections in 6 instances and in *P. vivax* infections in 20 instances. No exoerythrocytic forms were seen in the cultures.

REFERENCES

Dubin, I. N. 1947. Bodies suggesting exoerythrocytic forms of *Plasmodium vivax* in tissue culture. Proc. Soc. Exper. Biol. and Med., **65**: 154–156.

Hawking, F. 1945. Growth of protozoa in tissue culture; *Plasmodium gallinaceum*, exoerythrocytic forms. Trans. Roy. Soc. Trop. Med. and Hyg., **39**: 245–263.

Huff, C. G., and Coulston, F. 1947. A search for the pre-erthrocytic stages of *Plasmodium vivax* and of *P. cynomolgi*. Jour. Parasitol., **33**: (section 2): 27. (Abstract).

Shortt, H. E., and Garnham, P. C. C. 1948. The pre-erythrocytic development of *Plasmodium cynomolgi* and *P. vivax*. Trans. Roy Soc. Trop. Med. and Hyg., **41**: 785–795.

Shortt, H. E., Garnham, P. C. C., Covell, G., and Shute, P. G. 1948. The pre-erythrocytic stage of human malaria, *Plasmodium vivax*. British Med. Jour., **1**: 547–548.

Zuckerman, A. 1945. In vitro opsonic tests with *Plasmodium gallinaceum* and *P. lophurae*. Jour. Infect. Dis., **77**: 28–57.

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